LETTUCE (*Lactuca sativa* L.) ROOT MORPHOLOGY, ARCHITECTURE, GROWTH AND DEVELOPMENT IN AN AUTOTROPHIC CULTURE SYSTEM

By

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In loving memory of my father, Piero Nicola, who departed from us August 12, 1996. Dad did not have the chance to witness my academic achievement, but he has been an inspiration for all my life and will always be in my heart.

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Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
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LETTUCE (*Lactuca sativa* L.) ROOT MORPHOLOGY, ARCHITECTURE, GROWTH AND DEVELOPMENT IN AN AUTOTROPHIC CULTURE SYSTEM

By

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Studies on root morphology and architecture in an effort to improve growth rates could be more valuable if plant growth systems could be optimized for root studies. One objective of this research was to establish an autotrophic plant culture system to study daily changes in root growth and architecture as they are affected by alterations in the growth medium, light, temperature, and atmospheric changes. Root morphology and development of lettuce (*Lactuca sativa* L.) from radicle protrusion to 18 days after seeding (DAS) was described by utilizing this system. Differentiation between lettuce basal and lateral roots arising from the taproot was shown. Basal roots arose earlier than lateral roots. The final number of lateral roots was always greater than the number of basal roots.

Studies with a Photo-mixotrophic Whole Plant Culture (PWPC) system indicated that exogenous sucrose application to the medium enhanced root growth in lettuce seedlings 9 DAS, and the response varied according to genotype. Further studies indicated that lettuce seedlings preferentially use CO_2 to sucrose. Nitrogen at 120 mM concentration suppressed root branch formation compared to N at 60 mM. Root architecture was usually altered by environmental conditions without affecting plant biomass.

The statistical approach used to analyze the data collected demonstrated that analyses of plant growth in interaction with time should not be performed with univariate F tests, otherwise, consequent interpretations of the results may not be valid. The Greenhouse-Geisser P-value adjustment was used for statistical analysis of repeated measurements.

Genetic variability for rooting characteristics and response to enhanced carbohydrate resources varied among wild and cultivated lettuces. Incorporating specific rooting patterns into new cultivars could be an effective strategy to genetically select new superior lettuce biotypes. Reducing N application in the growth medium may be a valuable tool to regulate and enhance root growth and development in young lettuce seedlings. In addition,

enriching atmospheric CO_2 in controlled environments may be a valuable approach to increase early root growth.

CHAPTER 1
INTRODUCTION

Researchers generally concentrate on the physiology of the shoot and neglect the roots of plants, partly because roots are out of sight and are more difficult to study. However, their functions indicate that vigorous root systems are as essential as vigorous shoots for growth and development of healthy plants. In fact, the growth of roots and shoots is completely interdependent, and one plant part cannot develop effectively if the other fails (Kramer, 1983).

Root systems of terrestrial plants can serve an array of primary and secondary functions:

1) Acquisition and translocation of water and dissolved ions are a primary function of roots. This is considered to have been a key event in the evolution of terrestrial plants.
2) Capacity for structural support of upright development in plants is also considered an important function of root (Kramer, 1983). The significance of this role appears to have increased during the evolution of land plants, because the

earliest terrestrial species had poorly developed root systems (Collinson and Scott, 1987).

3) A storage role for roots is also indicated by the large amounts of reserves, such as carbohydrate, found in roots of carrot, beet, sugar beet and cassava.
4) Roots also function as sites of synthetic activity. In many plants, inorganic nitrogen is converted to organic compounds (aminoacids) in the root before being translocated to the shoot.
5) A further role of roots is the production of hormones essential to both the root and the shoot. Cytokinin synthesized in the root (root tip and cambial region) is essential to developmental activities in root meristematic tissue (Feldman, 1975; Itai and Birnbaum, 1996). Cytokinins are also translocated to the shoot via the xylem where cytokinesis is promoted (Salisbury and Ross, 1992). In addition, roots synthesize significant quantities of gibberellins, which are translocated to the shoot in the xylem sap (Skene, 1967). Further, abscisic acid (ABA) production can be induced by water stress in the root (Davies and Zhang, 1991; Itai and Birnbaum, 1996; Salisbury and Ross, 1992; Zhang and

Davies, 1987). Moreover, ethylene can be produced in any plant tissue (Itai and Birnbaum, 1996). Stress conditions such as waterlogging, O_2 deficiency, anoxia, or anaerobiosis, can induce accumulation of an ethylene precursor (1-aminocyclopropane-1-carboxylic acid, ACC) in the roots. This is due to an O_2 requirement for transformation of ACC into ethylene, which in turn alters whole plant growth and development (Abeles *et al.*, 1992; Bradford and Yang, 1981; Kawase, 1981). Auxin is produced in the root. Indole acetic acid (IAA), one of the natural auxins, is actively metabolized by all parts of the root (Itai and Birnbaum, 1996).

6) Roots also function as sites for rhizosphere associations between the plant and bacteria or other microorganisms that provide nutrients useful for plant growth. The N from nitrogen-fixing *Rhizobium* bacteria and P from mycorrhizal fungi are two examples.

Root growth, development and architecture are thus important aspects of seedling growth. Stresses that reduce root growth may injure the plant by reducing the volume and extent of soil exploration. The root environment is seldom optimum for growth (Atta Aly *et al.*, 1989; Leskovar, 1995).

Adverse conditions include drought, reduced gas exchange, increased CO_2 levels, reduced O_2 levels, deficient or imbalanced nutrient supply, toxic chemicals, mechanical impedance, non-optimum soil temperatures, and attack by pathogens and insects (Leskovar, 1995; Miller, 1986). If root growth declines due to stress, the supply of water and nutrients to the shoot may be reduced, with a subsequent reduction in shoot growth. However, conditions that limit photosynthesis can reduce shoot growth, limit assimilate translocation to the roots, and in turn, can limit root growth. Therefore, stress originating in either the root or shoot affects whole plant growth (Brown and Scott, 1984; Miller, 1986).

Studies relating the effects of the environment immediately after radicle protrusion can provide valuable insights into implications of root perturbation on early seedling growth and subsequent plant development. Indeed, Leskovar and Stoffella (1995) stated that "… Root study methods and statistical procedures need to be improved or developed to further understand seedling root morphology, architecture, and development. …" (p.1157).

One objective of the present research is to establish a plant culture system for lettuce (*Lactuca sativa* L.) in a translucent agar-like medium, enabling the daily observation of changes in root growth and developmental processes. The culture system will facilitate the characterization of rooting patterns of different genotypes of *Lactuca sativa* L. This analysis of young seedlings in response to exogenous carbon source will test the hypothesis that superior root growth potential may have been lost during the selection of improved commercial shoot quality in lettuce. Further development of an autotrophic culture system will be pursued, and a description of root morphology, growth and development from initial radicle protrusion to the formation of a young lettuce seedling will be presented as a subsequent objective.

The autotrophic culture system will be used in subsequent studies to address the hypothesis that lettuce seedlings can differentially respond to exogenous or endogenous paths of carbon delivery. Root architecture and growth will be characterized in seedlings provided with two sources of carbon, either via exogenous sucrose application in the growth medium or CO_2 in the atmosphere. Following goals of the present research will be to test whether CO_2 enrichment results in a translocation of carbohydrate to roots, affecting root architecture and development, and whether increased N concentration in the growth medium causes adaptive changes in the roots leading to changes in root architecture and development. A statistic procedure will be implemented in an attempt to increase the validity of the analyses of results obtained.

CHAPTER 2
LITERATURE REVIEW

Morphology and Development of Young Roots in Dicotyledonous Species

The root is the lower portion of a plant axis which generally develops below the soil surface. A root system consists of main axes and their branches: vertical axes or taproots, first-order branches and higher-order branches. The apical meristem of branching roots develops deep within the inner tissues (at the periphery of the vascular cylinder, Esau, 1977) in contrast to the buds of the shoot, which develop from outer tissues (outside the vascular cylinder, Fahn, 1982). Therefore, branching of roots is endogenous and that of stems exogenous (Esau, 1977; Fahn, 1982).

Generally, seedling root morphology differs between monocotyledonous and dicotyledonous species (Fahn, 1982; Leskovar and Stoffella, 1995; Sutton and Tinus, 1983; Zobel, 1975, 1986). Roots of dicotyledonous species are generally classified into 4 types (Klepper and Rickman, 1991; Stoffella et al., 1988; Zobel, 1975, 1986, 1996): radicle, adventitious, lateral and basal. The radicle will form the taproot (primary root), adventitious roots are initiated from the hypocotyl, lateral roots from the taproot, and basal roots from the "lower hypocotyl and upper tap root," according to Zobel (1986, p.957) or from the "basal part of the hypocotyl or shoot-root transition zone when pericycle cells do not originate in this area," according to Leskovar and Stoffella (1995, p.1153). Zobel (1986) indicated that initiation of basal roots is under different genetic control from initiation of lateral and adventitious roots. In fact, a double homozygote from a lateralless tomato mutant (recessive mutant called diageotropica, *dgt*) and an adventitiousless tomato mutant (recessive mutant called rosette, *ro*) originated roots in the hypocotyl and upper portion of the taproot. Assuming only 3 types of root, the double homozygote should have had only a taproot. Because these roots were genetically not lateral, nor were they adventitious, Zobel (1975) classified these roots as 'basal' roots.

Adventitious roots cannot be confounded with lateral roots for two main reasons. First, adventitious roots originate from the stem, while lateral roots originate from the taproot. Second, the former types originate from tissues other than the pericycle, while the latter type originates from the pericycle. Conversely, basal roots are not clearly classified with respect to their point of origin. Zobel

(1986) stated that he demonstrated that basal roots originate from the pericycle of the lower hypocotyl and upper taproot. Consequently, basal roots were not adventitious in anatomical origin, nor lateral or adventitious in genetic control of their initiation. Leskovar and Stoffella (1995) indicated that basal roots originate from a transition zone between the shoot and root.

In bell pepper seedlings, basal roots emerged prior to lateral roots (Stoffella et al., 1988) and only after full cotyledon expansion. In tomato seedlings, lateral roots emerged prior to basal roots (Aung, 1982). Weinhold (1967) described basal roots as arising acropetally (toward the shoot apex) from the germinating seedling, while lateral roots arose basipetally (toward the radicle apex). Charlton (1996) did not distinguish between lateral and basal roots, but he defined lateral roots as "roots derived from lateral endogenous primordia formed in preexisting roots" (p.149). The author said that lateral roots appeared at a relatively constant distance behind the tip of a growing root, that lateral roots initiated in rows or ranks, and that within each rank they appeared to initiate and emerge in acropetal sequence under normal conditions. Charlton (1996) reported that between the basipetally emerged lateral roots, particularly in dicots, additional laterals may arise for a long period in roots with secondary growth. Esau (1965) referred to these lateral roots as adventitious.

Micropropagation Technique: A Soilless Medium Growth System

Micropropagation is a method of vegetative propagation (Kozai, 1991a). Photoautotrophic micropropagation or simply autotrophic micropropagation is defined as a method of plant propagation based on photoautotrophic tissue culture with all carbon derived from CO_2 (Kozai, 1991a). In this type of culture, growth and development are largely influenced by environmental factors, which include light, CO_2, humidity, air flow speed, temperature, and O_2. However, research on whole seedling photoautotrophic micropropagation is fairly new, and furthermore, there is minimal research on the effects of the environment such as carbon supply, light, mineral nutrition, on the photoautotrophic growth and development *in vitro* of whole seedlings.

In conventional vegetative propagation using leaf cuttings or stem cuttings, the plant part grows in a substrate such as vermiculite, under mist irrigation system located in a greenhouse, in the absence of sugar in the medium (Kozai, 1989). Sugar can be used as a carbon or energy source for heterotrophy of the explant/plantlet *in vitro* (Kozai, 1989, 1991a, 1991c; Kozai et al.,1990, 1991b).

Generally, the explant does not have enough photosynthetic capacity to develop autotrophy from a young cutting (Kozai, 1991b). Recent research on *in vitro* environmental physiology and *in vitro* ecology revealed that most explants containing chlorophyll and plantlets grown *in vitro* have a photosynthetic capacity similar to plants grown *ex vitro* (Kozai, 1989). Eventually, explants and plantlets can develop autotrophically with physical environmental factors such as CO_2 and light *in vitro*. When this occurs, no added sugar is required for growth.

Seedling growth *in vitro* does not require a supply of growth substances as in the case of an explant or a cutting. In fact, the seed from which the plantlet originates provides all the substances necessary for early growth. Plantlets have autotrophic growth assuming they are provided sufficient CO_2. When using airtight vessels for growing photoautotrophic seedlings, the CO_2 concentration was often found to be as low as the CO_2 compensation point (less than 100 $\mu mol\ mol^{-1}$) during most of the photoperiod (Fujiwara et al., 1987; Kozai et al., 1992). Using loose caps or gas permeable film for capping the growth vessels resulted in a CO_2 concentrations often lower than 200 $\mu mol\ mol^{-1}$ (Kozai, 1991a) (which is lower than atmospheric concentration of 350 $\mu mol\ mol^{-1}$). During the dark period, CO_2 levels rose up to

3000-9000 µmol mol^{-1}, but remained low (100-200 µmol mol^{-1}) for the entire light period.

The Type of Carbon Sources for Plant Growth

Sucrose is readily used as a carbon source by plants after enzymatic hydrolysis (Avigah, 1982; Giaquinta, 1983; Smith, 1993). Exogenous sucrose has been reported to enhance plant growth (Avigah, 1982; Stoffella et al., 1988) and excised tomato roots (Chin and Weston, 1975; Weston, 1975). Kozai et al. (1987, 1988a, 1988b) found that explants of carnation, *Cymbidium* and potato cultured *in vitro* under conditions of CO_2 enrichment and high light intensity (150-250 µmol $m^{-2}s^{-1}$) produced plantlets, each having more than twice the mass of those cultured under conditions of low CO_2 and low light intensity (35 µmol $m^{-2}s^{-1}$). Results were similar regardless of sucrose concentrations in the culture medium. Plant growth was promoted without sucrose or with 1% sucrose in the medium. Net photosynthetic rates per leaf dry weight and per plantlet decreased with increased sucrose concentration in the medium (Kozai, 1989).

Growth and development of *Brassica campestris* L. plantlets cultured in vessels were similar to those of seedlings cultured under the same *in vitro* environmental conditions (Kozai et al., 1991b). The growth and development of plantlets and seedlings were greater for treatments with a higher number of air changes in the vessel per hour and a higher photosynthetic photon flux regardless of the sucrose concentration in the culture medium. Low CO_2 in the vessel with a lower number of air changes per hour reduced the net photosynthetic rate of plantlets and seedlings in the vessel, affecting growth and development of both plantlets and seedlings. Kozai et al. (1988c) reported that growth of tobacco seedlings grown *in vitro* was improved by CO_2 enrichment, high PPF and sucrose-free medium. Growth was repressed by CO_2-non enrichment, low PPF and sucrose-free medium. However, growth of seedlings was restricted if sucrose was absent from the medium in relatively airtight vessels where photosynthesis would presumably have been limiting (Kozai, 1991a).

The Role of Sucrose in Plant Growth

Sucrose is a source of carbon skeleton for use in biosynthesis, and is a substrate during respiration processes to produce the energy for plant growth (Farrar and Williams, 1991). Recently, sucrose has been hypothesized to also have a regulatory role on gene expression, providing a mechanism for adjustment to environmental and/or developmental changes that alter photosynthate supplies (Jan and Sheen, 1996; Koch, 1996; Koch et al., 1996; Kovtun and Daie, 1995). Other morphogenetic processes have been attributed to be controlled by sucrose, such as induction of flowering (Bernier, 1988; Bernier, 1996; Bernier et al., 1993; Corbesier et al., 1996), differentiation of the phloem (Aloni, 1987; Jeff and Northcote, 1967; Wetmore and Rier, 1963; Wright and Northcote, 1972), initiation of root meristems (Street, 1969; van't Hoff et al., 1973) and initiation of roots in callus (Farrar and Williams, 1991; Wright and Northcote, 1972).

Changes in photoperiod altered the fluxes and levels of nutrients such as sucrose and Ca^{2+} in *Sinapis alba* (Bernier et al., 1993; Pryke and Bernier, 1978). Along other chemical changes, including some hormones, increased sucrose levels induced flowering (floral signaling system). In *Lolium temulentum*, a long-day requiring plant, an increase of sucrose did not appear to be part of the floral signaling system (King and Evans, 1991). However, Bernier et al. (1993) reported that sucrose and cytokinins seem to be involved in the floral signaling system of *Xanthium strumarium*, a short-day plant.

Several lines of evidence support the hypothesis that the path of C entry into a plant system or organ can alter its effect at both the substrate and signaling levels. The influence of carbon supply on plant growth and respiration can differ with the source and path of delivery for this resource. Correlations have been reported between respiration rate and carbohydrate content of roots, which have been interpreted as indicative of substrate limitation in respiration (Farrar and Williams, 1991). Farrar and Williams (1991) suggested viewing this relationship with caution, hypothesizing that only cytosolic sugars would be expected to correlate with respiration rate, as was observed in their studies with barley roots. Nevertheless, these researchers pointed out that when exogenous sugars are supplied at high concentrations, further stimulation of respiration is minimal. In contrast, growth and respiration increased when photosynthetic input was elevated by raising light intensity or partial pressure of CO_2. Respiratory responses to exogenous sucrose feeding can thus differ from those to CO_2 enrichment, and these differences in turn may be related to altered effects on growth and morphology.

Sugar-responsive gene expression can differ depending on whether sucrose is delivered directly to chloroplasts (Sheen, 1990), to leaves (Van Oosten and Besford, 1994), or to seedlings in culture (Brusslan and Tobin, 1992). These studies indicated that sugars can either repress or induce the expression of different genes that encode key enzymes of carbon fixation and metabolism, depending on the experimental system.

Kovtun and Daie (1995) found that sugar beet (*Beta vulgaris* L.) seedlings grown with either 90 or 150 mM sucrose, 90 or 300 mM glucose increased growth rate, photosynthetic rate, and leaf sugar levels, regardless of which sugar was added to the agar-media. The steady-state level of transcripts increased for the small and large subunits of ribulose-1,5 bisphosphate carboxylase/oxygenase (Rubisco) and the cytosolic fructose-1,6 bisphosphatase. The activity of sucrose phosphate synthase increased in the presence of sugars, but the fructose-1,6-bisphosphatase and Rubisco activities remained unchanged. Related evidence suggested that exogenous sugars may have had minimal or no osmotic effects in whole plant experiments since similar growth rates were measured with an osmotically-adjusted medium (300 mM 3-O-methyl glucose) and with sugars. Results from the Kovtun and Daie (1995) studies suggested that, in addition to serving as molecular signals, sugars play important physiological roles in the development of the export capacity to sink tissues. In sugar beet seedlings, the addition of sugar to the culture medium caused accelerated leaf growth, development and sink-to-source transition.

The Relationship between Carbon and Nitrogen

The activities of carbon and nitrogen assimilatory processes are closely related to rates of plant growth and development. The constancy of this association has led modelers of physiological responses to explain whole-plant growth and coordinated growth between the shoot and root predominantly in terms of carbon and nitrogen interaction (Thornaley, 1976; Wann and Raper, 1979). It is not surprising, therefore, that disruption in the supply of carbon or nitrogen can result in marked changes in the assimilation of the other. In the case of nitrogen stress, for example, two distinct changes in carbon utilization are commonly observed: a) starch accumulates in leaves (Ariovich and Cresswell, 1983; Radin and Eidenbock, 1986; Robinson and Baysdorfer, 1985; Wilson, 1975) and b) a larger portion of available carbohydrate is translocated from leaves into the root system, resulting in a increase of root:shoot ratio (Brouwer, 1962; Ingestad, 1979). The responses imply a general decline in carbohydrate utilization efficiency within the leaf canopy (Rufty et al., 1988). Increased translocation of carbohydrate to roots with nitrogen stress is probably related, at least in part, to a decreased rate of leaf canopy development (Rufty et al., 1988).

Changes in the relationships between carbon and nitrogen assimilatory processes can also occur when the carbon supply is increased. Doubling of the atmospheric CO_2 concentration, which is expected to occur by the middle of next century (Murray, 1995), has been reported to increase crop yield (Sonnewald et al. 1996). Sonnewald et al. (1996) reported that the initial response to elevated CO_2 in the atmosphere leads to an approximately 50% increase in leaf photosynthesis. Elevated atmospheric CO_2 increases photosynthetic efficiency in terrestrial C_3 plants because the present CO_2 level is insufficient to saturate the Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and the increased CO_2 competitively inhibits Ribulose-1,5-bisphosphate oxygenation and photorespiration (Long et al., 1996). However, following this response an acclimation effect occurs, and the rate of photosynthesis declines again in many species (Sonnewald et al., 1996), mainly due to a decline of Rubisco activity.

Long et al. (1996) hypothesized that if an increased atmospheric CO_2 concentration leads to an increased net efficiency of photosynthetic CO_2 assimilation, then an increase in net photosynthetic carbon gain should be expected regardless of whether other requirements for plant growth, such as light and nitrogen, are limiting or saturating. This is because of an increased efficiency of light energy conversion into assimilated carbon rather than an increased supply of a co-limiting substrate.

Long et al. (1996) studied the long-term effect of CO_2 on stimulation of photosynthesis on ryegrass (*Lolium perenne*) grown in pots and in the field, with both low and high nitrogen supplies. An acclimatory loss of Rubisco activity and protein was found at both nitrogen levels, but more when nitrogen was limiting. Johnson et al. (1996) grew seedlings of two species of *Pinus* (*Pinus ponderosa* Laws. and *Pinus taeda* L.) with elevated CO_2 in the atmosphere and with or without N in the growing medium. These researchers reported (Johnson et al., 1996) that the N deficiency precluded plant response to CO_2 in *Pinus ponderosa* and reduced plant response in *Pinus taeda*. The elevated CO_2 and N levels caused a plant growth increase in the field for *Pinus ponderosa*, through an increase of root biomass. Unfortunately, no values of nitrogen and CO_2 levels were given, but the authors concluded that nitrogen can be a limiting factor for plant response to CO_2, because of plant increased N demand upon elevation of CO_2 availability.

The effect of elevated CO_2 in the atmosphere has been often studied by scientists, but mostly on shoot growth. Since consequences of CO_2 in the air affect photosynthesis rate, and because nitrogen supply may become a limiting factor during increased photosynthesis rate, increasing nitrogen supply in the rooting system may or may not increase plant growth. More importantly, an increase in nitrogen supply in the rooting medium could change root morphology and architecture to allow roots to adapt to the changed conditions.

Lynch (1995) reported that in most natural ecosystems water and nutrients are limiting factors in plant growth. Lynch stated that these limiting factors may limit yield in most agricultural ecosystems, and in the United Stated and other industrialized nations, intensive irrigation and fertilization have generated environmental problems. Therefore, a subject of considerable interest in agriculture and ecology is the acquisition of soil resources by plant root systems.

Nitrate in sandy soils can be highly mobile, and it is efficiently absorbed at the root surface by the plant. The uptake of nitrate remains largely unaffected by changes in its concentration in the soil solution around the roots until it is virtually depleted (Burns, 1980; Edwards and

Barber, 1976). Plants tend to increase their nitrate inflow rate (i.e. the rate of uptake per unit length of root) through the exposed roots, with up to threefold increases observed for different crops when a highly localized nitrate supply was imposed (Burns, 1991; Drew and Saker, 1975; Edwards and Barber, 1976; Robinson and Rorison, 1983). Drew et al. (1973) stated that plants increased root growth in the nitrate-rich zones, often at the expense of root growth in other regions. Since nitrate inflow rates cannot be increased without limit when the proportion of roots exposed to nitrate is reduced, Burns (1991) suggested that N uptake must become dependent on root length when the number of absorbing roots is small, and that the minimum amount of roots required to sustain N uptake will depend on the N demand of the plant. Imsande and Touraine (1994) stated that to cope with variations in mineral concentrations in soil, plants have evolved mechanisms to regulate the activity of the uptake system in such a way that the net intake of a nutrient depends on the plant need for the given element rather than its concentration in the medium.

Nitrate uptake is of special interest in mineral nutrition because nitrate is absorbed at a relatively high rate by the plant (Imsande and Touraine, 1994). However, while there are many studies on the effects of nitrogen stress on plant growth, very few have focused on an excess of nitrogen on plant growth, particularly on root morphology and architecture. Differences in soil root distribution between plant species can provide clues regarding spatial and temporal differences in the uptake of water and nutrients (Klepper, 1987; Parris and Bazzaz, 1976).

Measurements of root biomass and root length are typically used to describe root distribution in the soil profile, but analysis of other aspects of root system structure, such as root morphology and architecture, can improve the understanding of water and nutrient extraction from soil (Fitter, 1985). A major difficulty in studying root systems in the soil is the extraction of fine roots and measurement of architectural parameters. Fitter and Stickland (1991) and Lynch and van Beem (1993) suggested that the architecture of a root system may have ecological implications for uptake of water and nutrients from soil. Fitter (1986, 1987) suggested that, in general, plants with a more herringbone-like distribution of roots, that is, with branches mainly along the central root axis, may occur under low soil resource availability, whereas secondary branches increase when resources are present in abundant supply, thereby increasing acquisition of water and nutrients.

Origin of Lettuce (*Lactuca sativa* L.)

Cultivated lettuce was domesticated several thousand years ago. Jackson (1995) and Bianco (1990) stated that it is probably a close relative of *Lactuca serriola* L., a Mediterranean annual species native to dry habits. Bianco (1990) reported that the probable origin of lettuce is the Middle East, and that the first appearance of some kind of documentation is a canvas, dated 3000 BC, found in an Egyptian tomb. Ancient Greeks and Romans ate lettuce (Bianco, 1990). The first description of crop cultivation regarding lettuce is dated 300 BC by Theophrastus. Subsequently, Plinium reports on lettuce were found. In the Middle Ages, reports were not noted, whereas in the 17th century there were technical reports of cultivation. Romaine lettuce is thought to have originated in Italy (Bianco, 1990) where it has been known since the Middle Ages. Lettuce was exported into France by Rabelais in 1537, and Christopher Columbus brought some types of lettuce to the new world. In Brazil lettuce was cultivated prior to 1650 and in Haiti in 1865.

High variability among species, lines and varieties exists today because of natural mutations and/or hybridization with *Lactuca serriola* L. (Bianco, 1990), the

Mediterranean species, even though *Lactuca sativa* L. grows spontaneously in Siberia.

Estimation of worldwide production of lettuce is around 5 million tons per year (Bianco, 1990), with the United States the major producer (1,840,000 t) followed by China.

Cultivated lettuce has been bred for desirable growth and head characteristics in vegetable production systems with high inputs of nutrients and frequent irrigations (Rowse, 1974).

Root Systems in Lettuce

Lettuce root systems have been described as early as 1927 by Weaver and Bruner (1927). The authors described the "common lettuce of the garden (*Lactuca sativa crispa*) as a tall, leafy, annual herb with a milky juice" (p.320). The term "milky juice" can easily be related to the probable origin of the Latin name *Lactuca*, which appears to derive from the word *lactucus*, i.e., "rich in milk" (Bianco, 1990, p.270).

To describe root system development, Weaver and Bruner (1927) planted 'Early Prize Head' lettuce seeds in soil. Early root growth has been described to take place rapidly, with lateral branches appearing on the first 25 to 40 mm of the taproot, in conditions very favorable for growth, 6 days after cotyledons unfolded. These first branches were described as arising horizontally and very near to the soil surface. Thirty-eight days later, at the first examination in the field, the plants were 75 mm tall, the taproots were 5 mm in thickness near the soil surface and gradually tapered to 1 mm towards the root tip. Taproots were course and grew almost vertically downward for 500-700 mm in depth and unbranched in the last 150 to 200 mm. Branches arose on two opposite sides of the taproot, mostly in the first 300 mm of soil. Sixteen roots were counted arising from 25 to 50 mm below the surface, followed by 7 to 14 roots arising every 25 mm of taproot length for about 150 mm. After that fewer, short and unbranched primary branches were found closer to the taproot tip. The authors reported that in the surface 150 mm of soil, many of the roots ended within 5 mm of the taproot, whereas others extended horizontally 100 to 200 mm, and a few had a length of 400 to 500 mm. Branches of the third order were noted on the oldest roots, sometimes reaching a length of 17 mm, while below 100 mm, the lateral roots were unbranched.

Jackson (1995) hypothesized that *Lactuca sativa* (cultivated type) and *Lactuca serriola* (wild type) differed in root architecture, on the basis of timing and distribution of water and nutrients in the soil, and that inadvertent selection has occurred for root characteristics in *L. sativa*, that would contribute to rapid growth and shoot uniformity under cultivation practices that utilize large amounts of water and nitrogen. The author conducted a series of experiments addressing the genetic and management controls on nutrient cycling in agricultural systems. He grew seedlings of *Lactuca sativa* cv. Salinas and *Lactuca serriola* for 5 to 6 weeks in a greenhouse. The studies were conducted using either pots or cylinders filled with coarse-textured soil. Root biomass allocation was very similar at 4 to 6 weeks after transplanting, but root architecture differed. In the top zone along the taproot (0 to 50 mm), cultivated lettuce tended to produce more lateral roots (probably this type of root is what other authors call basal root), a greater total root length, and more external links [(segments that originate at a branch point and end in a meristem, referred to as *magnitude* by Fitter (1996)] than the wild lettuce. Conversely, from the 50 to the 550 mm zone of the taproot, wild lettuce produced more laterals than *L. sativa*. Jackson (1995) concluded that inadvertent selection may have occurred in the breeding of cultivated lettuce varieties. These selections occurred for increased root growth in the surface zone where water and fertilizers were applied and for greater plasticity in construction of root segments, which may maximize the efficiency of exploitation of soil moisture and nutrients.

Conclusions

The production of a primary root system, i.e. the primary branching from the radicle, is an important phenomenon in growth and survival of a plant (MacIsaac et al., 1989). A primary root system increases the surface area available for the uptake of water and mineral elements. In addition, with its architecture, a primary root system gives support of the shoot to the substrate or soil.

Root architecture can be very important in plant productivity. Many of the soil's resources are unevenly distributed, or are subjected to localized depletion by the roots, so that the spatial distribution of the root system can determine the capacity of a plant to exploit those resources and to respond to localized availability of them (Lynch, 1995). Patches of localized nutrients in the soil, such as phosphorus, may be available to the root system only if roots have the potential to reach the patches. Gradients in temperature, O_2 and CO_2 content, pH, and water availability, commonly occur in soils. Soil gradients, such as soils richer in nutrients at the surface or soils which are more subject to drought and/or temperature extremes, may also occur. Seasonal gradients in soils change the mobility of mineral nutrients such as nitrogen. Warmer temperatures improve uptake of nitrate by the root, whereas cooler temperatures favor uptake of ammonium (Mengel and Kirkby, 1982). Exploitation by the root system of soil resources may consume more than 50% of the available photosynthate in mature plants (Fogel, 1985).

The architecture of a root system determines its exploration of the soil for resources and the plant capacity to dynamically respond to the localized availability of soil resources through meristematic activity (Lynch, 1995). Root architecture also has important implications for the construction costs associated with exploration of a given volume of soil (Fitter, 1996). Therefore, the importance of root architecture in plant productivity is related to the need for exploiting a very heterogeneous environment, and in doing so, high construction costs of the root system sustained by the plant cannot be wasted.

Adventitious roots have not been reported to occur in lettuce seedlings. Whether a distinction between basal and lateral root formation in lettuce seedlings exists has not been investigated. Basal roots have been reported to be produced by several species, such as mungbean (*Vigna radiata* L.) (Leskovar and Stoffella, 1995), bell pepper (*Capsicum* *annuum* L.) (Stoffella et al., 1988), tomato (*Lycopersicon esculentum* Mill.) (Zobel, 1975) and beans (*Phaseolus vulgaris* L.) (Stoffella *et al.*, 1979). The literature, however, does not report to our knowledge any description of root architecture in lettuce, besides the works mentioned above. Understanding how the root architecture in lettuce seedlings is structured may be valuable to improve root growth.

Minimal information on root architecture exist since it is difficult to observe, quantify and interpret. Field methods to study root growth enabling direct observations of the roots without their removal form the soil have not been found (Mackie-Dawson and Atkinson, 1991). Observation methods to study root architecture use a viewing surface inserted into the soil. However, these methods allow observations only on an area of the root system. If a whole viewing surface could be developed, these observation methods could represent a major advance to study the root system.

Studies on root morphology and architecture have attracted research in the field of forestry and fruit crops, since these species have a long life span and high dependence on the below-ground conditions, e.g. water supply, fertility, soil physical property, aeration.

Research on vegetable crops has focused mainly on shoot growth, possibly since shoots are considered the main source for maximization of the edible part of the plant (even when the edible part of the plant is below ground, i.e. tubers, roots or crowns). While we now know quite extensively early shoot growth and development, we still need a lot of effort to understand root growth.

CHAPTER 3

SUCROSE ENHANCES INITIATION OF LATERAL AND BASAL ROOTS IN LETTUCE GROWN IN PHOTO-MIXOTROPHIC WHOLE PLANT CULTURE (PMWPC)

Introduction

The value of root development studies could be increased if experimental approaches could distinguish between differential growth and responses to external stimuli of lateral and basal root systems. If the two types of roots can be distinguished at the level of morphology and development then the effects of environmental conditions can be examined relative to initiation and development of one type of root over another. In addition, differences between the types of root can be taken into account when investigating root function, growth, or development (Zobel, 1986). This information has a distinct advantage over dry matter accumulation alone, since enhancement of root mass can simply indicate translocation of assimilates toward the taproot(s), which then lignify and suberize. This process in turn can decrease the absorbing capacity of the root for nutrients from the soil, especially if the young primary tissues of the root branches are affected.

Basal roots differ from the three more classic types (taproot, lateral and adventitious roots) in terms of morphology, gene expression, and development (Zobel, 1996; Chapter 5). This suggests possible differences in function as well. Variation in root growth between species was indicated by Seiler (1994) to be an important factor determining differences in drought tolerance among genotypes. Early, rapid root growth and branch root development were suggested to confer an adaptive advantage in more efficient utilization of soil water. Root elongation can be advantageous to plants in drying soil, and may be particularly important for seedling establishment. Growth of new plants is restricted largely to surface soil layers which are vulnerable to drying (Sharp et al., 1988). Zobel (1996) reported that the most drought tolerant species had the most deeply penetrating root systems. For instance, xerophytic woody perennials produced very deep penetrating root systems. Eshel and Waisel (1996) suggested that the major function of basal roots was to exploit the most fertile portions of agricultural soils more efficiently than lateral roots. In addition, Bole (1977) found that basal roots of rape (*Brassica campestris* L.) were able to absorb phosphorus more efficiently than lateral roots.

Limited research has been conducted on the variability of root systems among genotypes of crop species (Seiler, 1994). Utilization of wild progenitor species of lettuce may have the potential to enhance root growth characteristics due to the different adaptive strategies of the wild species versus the cultivated types.

Lettuce (*Lactuca sativa* L.) in Florida is produced mainly in organic soils (Guzman et al., 1992). The subtropical climate of the region favors many lettuce diseases, such as lettuce mosaic virus (LMV), bidens mottle virus (BMoV), corky root rot (CRR) (caused by *Rhizomonas suberifaciens* van Bruggen et al.) and lettuce downy mildew (caused by *Bremia lactucae* Regel).

New lines of Mediterranean lettuce were brought in Florida as Plant Introduction (PI) lines when the Florida lettuce breeding program initiated in the 1970s. These were crossed with American cultivated varieties in order to breed new cultivars resistant to LMV and also adapted to Florida conditions (Guzman et al., 1992). Cultivated lettuce, therefore, has been bred for disease tolerance, desirable yield and head quality characteristics. In intensive vegetable production systems there can be a high input of nutrients and frequent irrigations (Rowse, 1974). For these reasons genetic selection may have favored lines which had increased root growth in the surface zone of the soil, maximizing the root system efficiency of exploitation of soil moisture and nutrients at this soil level (Jackson, 1995). Investigations of root architecture in wild progenitor and selected genotypes of lettuce allow one to compare their characteristics and rooting patterns.

Sucrose provides carbon skeletons for plants after enzymatic hydrolysis (Avigah, 1982; Giaquinta, 1983; Smith, 1993). Exogenous sucrose in the growth medium has been reported to enhance plant growth in sugar beet (*Beta vulgaris* L.) seedlings (Kovtun and Daie, 1995), pepper (*Capsicum annuum* L.) seedlings (Stoffella et al., 1988) and excised tomato (*Lycopersicon esculentum* Mill.) roots (Chin and Weston, 1975; Weston, 1975). Sugar supplies in the plant system can alter enzyme activities, metabolism and development (Koch, 1996). In addition, sugars not only function as a carbon substrate for plant growth but they have been indicated as affecting the expression of genes for carbon fixation and metabolism (Sheen, 1990, 1994). Plant carbohydrate depletion up-regulated genes for photosynthesis, reserve mobilization and export processes from sink to sources tissues (Koch, 1996). Conversely, carbohydrate abundance up-regulated genes for storage (starch and storage protein accumulation) and utilization (respiration) in sink tissues. Therefore, carbon balance or exogenous sucrose in a seedling could markedly influence plant development through its influence on genes for carbon storage and utilization.

In this research Photo-Mixotrophic Whole Plant Culture (PMWPC) (Kozai et al., 1991a) was used to address the hypotheses that exogenous sucrose feeding can enhance root growth, that sucrose can differentially affect the formation of lateral and basal root types in lettuce seedlings, and further, that developmental effect of sucrose might vary with genotype.

Materials and Methods

Transparent gel 'Phytagel' (Sigma Chemical Co., St. Louis, MO) (2.5 g l^{-1}) was used as a medium for growing and evaluating root morphology and development of lettuce seedlings from radicle protrusion to 9 days after seeding (DAS). The basal nutrient medium contained inorganic salts as per Murashige and Skoog (1962) (Table 3-1) with or without 30 g l^{-1} (90 mM) of sucrose. The solution pH was adjusted to 6.5 with 1 N NaOH prior to autoclaving. Test tubes (15 x 2.5 cm) were prepared by adding 30 ml of medium and autoclaving them for 35 min at 130° at 1.1 kg cm^{-2} pressure (15 psi). Tubes were closed with loose caps and not

Table 3-1. Composition of Murashige and Skoog (1962) medium for tissue culture

Major elements				Minor elements		
Salts	mg/l		mM	Salts	mg/l	µM
NH_4NO_3	1650	N	41.20	H_3BO_3	6.2	100
KNO_3	1900	N,K	18.80	$MnSO_4 \cdot 4H_2O$	22.3	100
$CaCl_2 \cdot 2H_2O$	440	Ca	3.00	$ZnSO_4 \cdot 4H_2O$	8.6	30.0
$MgSO_4 \cdot 7H_2O$	370	Mg,S	1.50	KI	0.83	5.0
KH_2PO_4	170	P,K	1.25	$Na_2MoO_4 \cdot 2H_2O$	0.25	1.0
Na_2EDTA	37.3	Na	0.20	$CuSO_4 \cdot 5H_2O$	0.025	0.1
$FeSO_4 \cdot 7H_2O$	27.8	Fe	0.10	$CoCL_2 \cdot 6H_2O$	0.025	0.1

sealed. The medium was subsequently solidified at ambient temperature.

Lettuce types were classified as crisphead, butterhead, cos or romaine, leaf, stem and "Latin" lettuce (Guzman and Zitter, 1984). The latter resembles a butterhead lettuce in leaf texture, but is greener in color and produces soft heads or mostly leaves in rosette form. In the present study, several cultivars of lettuce were selected from the Florida breeding program as well as several standard cultivars. 'Floribibb', 'Dark Green Boston' and 'Everglades' are butterhead types of lettuce; 'Parris Island', 'Tall Guzmaine', 'Floricos 83' and 'Valmaine' are romaine types. The plant introduction lines 'PI 251245' and 'PI 68288' are "Latin" types, from the wild *Lactuca serriola* L., of South Mediterranean origin (Guzman and Zitter, 1983). Some of the cultivars had the LMV resistance because they contained a wild type lettuce of Egyptian origin resistant to LMV. In the case of 'Floricos 83' and 'Tall Guzmaine' the wild type was 'PI 68288' (Guzman, 1986). Some others possessed LMV resistance from 'Gallega' (a "Latin" lettuce), such as 'Dark Green Boston', 'Everglades' and 'Floribibb'. 'Valmaine' is considered to carry wild genes not only because its pedigree contains the wild type line 'PI 251245', but also because one of its parents is a wild type from Turkey, 'PI 167150'

(Guzman and Zitter, 1983). The 10 genotypes used in the present study could also be distinguished by other disease resistance characteristics, as well as tolerance to thermodormancy. The cultivated types of lettuce were adapted to semitropical Florida organic soils, while the two wild types were adapted to arid Mediterranean soils.

To initiate cultures, seeds (Petoseed Co., CA) were surface-sterilized by soaking them in a solution of 2% NaOCl and 0.1% Tween 20 for 20 minutes, rinsed five times in sterilized-deionized water, and placed in a Petri dish with filter paper no.3 (Whatman International Ltd. Maidstone, England) moistened with sterilized-deionized water. Germination progressed for 24 hr at 20-22°C under continuous fluorescent light (Osram Sylvania Inc., Danvers, MA, 50 μmol $m^{-2}s^{-1}$ PPF). When radicle length reached approximately 2 mm, one seedling was transferred to each vessel and placed at the center of the gelled medium surface. The seedling was carefully inserted into the medium leaving the upper hypocotyl above the gel surface. This technique provided an excellent nondestructive method for studying root architecture (Stoffella et al., 1988).

Test tubes were arranged in racks covered with a layer of aluminum foil around the medium to block light in the root zone. The racks were placed in a growth chamber under cool-white fluorescent tubes (Osram Sylvania Inc., Danvers, MA; 16 tubes of 160 W each) and incandescent bulbs (Osram Sylvania Inc. Danvers, MA; 12 bulbs of 67 W each) delivering 350 µmol $m^{-2}s^{-1}$ plant photon flux (PPF) at the plant level. Plants were grown under a cycle of 16 hr light and 8 hr dark. Temperature in the growth chamber was kept constant, thus maintaining an internal vessel temperature of 25/20°C ± 1°C (day/night).

The numbers of basal and lateral roots of each seedling were counted daily for 9 days following seeding, by visual observation through the glass growth vessel. Taproot length was measured with an approximation of ±2 mm by placing a ruler parallel to the taproot growing direction along the test tube. Total root number (lateral + basal roots) was computed at any given point in time. At the end of the growth period, seedlings were gently removed from the vessel upon separating the gel in the tube with a pair of forceps. Roots and shoots were washed by hand under running water and dry weights were obtained after oven drying of each sample for 96 hr at 60°C.

Each treatment involved ten observations, and the experimental design consisted of a split-plot with genotype as the main plot treatment and sucrose as the sub-plot treatment. The experiment was repeated twice and each was treated as a replicate. Data were analyzed using a SAS statistical system (SAS Institute Inc., 1989). All measures were taken daily and data submitted to analysis of variance of repeated measurements to investigate plant growth responses over time (Littell, 1989; Chapter 8). Validity of the F test for the main effects of genotype and sucrose and their interaction did not require the Huynh-Feldt (1970) condition of the repeated measurements (Littell, 1989; Chapter 8), consequently the univariate F test was considered correct (Table 3-2). All χ^2 tests of Mauchly's criterion were highly significant (Appendix A8-16), consequently the Greenhouse-Geisser (G-G) adjusted P values were used for all interactions with time (Greenhouse and Geisser, 1959). Data of the growth trend were plotted over time for each genotype and each sucrose level.

Trend analyses were performed by considering three main growth phases: 1) phase of initial growth, from the protrusion of lateral and/or basal roots (4 DAS to 5 DAS); 2) phase of expected response to treatments (5 to 6 DAS); 3) phase of continuation of growth (6 to 9 DAS). In Phase 3 slopes of the line segments were computed and analyzed. Data from measurements taken at the termination of the experiment were submitted to the analysis of variance.

Results and Discussion

Root Growth and Development 4 to 9 Days After Seeding

In most of the lettuce genotypes, primary - lateral and basal - root branches emerged between 4 and 5 DAS, as they did for other species such as sunflower (*Helyanthus annuus* L.) (Seiler, 1994), guar [*Cyamopsis tetragonoloba* (L.) Taub.] (Stafford and Michael, 1990) and cotton (McMichael et al., 1987). Lateral roots began to emerge 4 DAS in 'Everglades', 'Floricos 83', 'Valmaine', 'PI 251245' and 'PI 68288' regardless of whether seedlings were sucrose-fed or not (Figure 3-1a). Lateral roots of 'Floribibb' emerged 4 DAS when sucrose was added to the medium (Figure 3-1b). With few exceptions, lateral roots emerged at 5 DAS in the remaining genotypes. For 'Musette', however, plants in sucrose-free medium did not form lateral roots until 6 DAS, and the same was observed for 'Parris Island Cos' and 'Tall Guzmaine' when grown in sucrose-amended medium.

Basal roots did not form in nonsucrose-fed seedlings of 'Floribibb', 'Dark Green Boston' and 'Musette' (Figure 3-2a). In other seedlings without sucrose in their medium, basal roots began to emerge at 4 DAS, as in 'Parris Island Cos', Floricos 83', 'Valmaine' and 'PI 68288'. When sucrose was added to the medium, basal roots emerged 4 DAS in

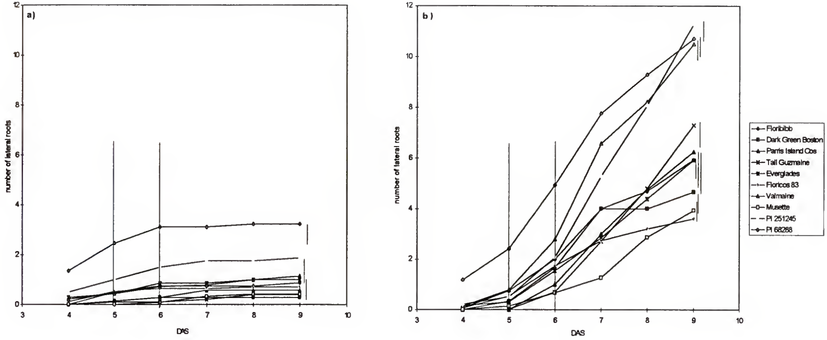

Figure 3-1. Number of lateral roots in lettuce seedlings grown from 4 to 9 DAS without (a) and with (b) sucrose in the medium. (Vertical lines at 5 and 6 DAS indicate separation of the phases of growth. Bars at 9 DAS indicate standard errors).

'Floribibb', 'Valmaine' and 'PI 251245' (Figure 3-2b). In the remaining genotypes basal roots generally emerged at 5 DAS. Sucrose-fed seedlings of 'Parris Island Cos' and 'Dark Green Boston' seedlings the first basal roots emerged at 7 and 8 DAS, respectively.

The presence of sucrose in the medium increased lateral, basal and total root numbers and taproot length with an interaction with genotype and time (Table 3-2). The enhanced root growth due to exogenous sucrose application might be linked to the influence of sucrose as a carbon source, and/or the effect of other morphogenic signals which involve enzymes of carbon fixation and metabolism (Koch, 1996; Kovtun and Daie, 1995; Chapter 6).

Wild types of lettuce had enhanced lateral root initiation without sucrose compared to the other genotypes, and responded significantly to the addition of sucrose in the medium (Figures 3-1a and 3-1b). This finding extends studies of Jackson (1995), who reported the wild type of lettuce (*Lactuca serriola*) formed more lateral than basal roots compared to cultivated types. 'Valmaine' seedlings grown with sucrose in the medium had an increased number of lateral roots compared to the other cultivars, and produced the same number of lateral roots as the wild types 9 DAS. The wild type progenitors in the background of 'Valmaine'

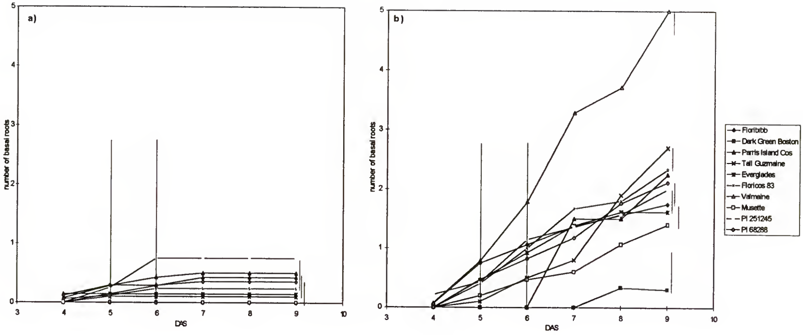

Figure 3-2. Number of basal roots in lettuce seedlings grown from 4 to 9 DAS without (a) and with (b) sucrose in the medium. (Vertical lines at 5 and 6 DAS indicate separation of the phases of growth. Bars at 9 DAS indicate standard errors).

Table 3-2. Univariate analysis of variance of repeated measures.

		Lateral Roots		Basal Roots		Total Roots		Taproot Length	
Source of variation	DF	Univariate F Test	G - G Adjustment	Univariate F Test	G - G Adjustment	Univariate F Test	G - G Adjustment	Univariate F Test	G - G Adjustment
Block	1								
Genotype	9	0.0001		0.0135		0.0004		0.0143	
Genotype (error a)	9								
Sucrose	1	0.0001		0.0002		0.0001		0.0008	
Sucrose * Genotype	9	0.0062		0.2640		0.0140		0.4953	
Sucrose * Block(Cv) (error b)	9								
Sampling error	189								
Analysis over time									
Time	5	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	<0.0001	0.0001
Time * Block	5	0.0001	0.0008	0.0079	0.0378	0.0001	0.0019	0.0005	0.0096
Time * Genotype	45	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Time * Genotype * Block	45	0.0277	0.1172	0.0446	0.1164	0.0021	0.0378	0.0001	0.0020
Time * Sucrose	5	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Time * Genotype * Sucrose	45	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0040
Error	990								

could have contributed to its capability to form more lateral roots compared to the other cultivars. 'Tall Guzmaine', a descendent of 'Valmaine', crossed with other lines for CRR tolerance, had more lateral roots 9 DAS than the other cultivars, but did not produce the same number of lateral roots as 'Valmaine'. 'Floribibb' and 'Floricos 83' are descendants of 'Valmaine', however did not show increased lateral root formation in response to sucrose in the medium. At 9 DAS 'Floricos 83' had a similar number of lateral roots as 'Musette' and 'Dark Green Boston'.

In most of the genotypes, basal root formation was enhanced by the presence of sucrose in the medium in interaction with time (Figures 3-2a and 3-2b). At 9 DAS, sucrose-fed 'Valmaine' seedlings had the greatest number of basal roots of all genotypes. 'Musette' and 'Floribibb' did not produce any basal root without sucrose, but showed basal root formation with sucrose added to the medium. 'Dark Green Boston', which did not form basal roots without sucrose, had the least number of basal roots 9 DAS.

Total root number was affected by an interaction between genotype, sucrose and time (Table 3-2). Sucrose enhanced total root formation of all genotypes (Figure 3-3b). At 9 DAS, sucrose-fed PI lines and 'Valmaine' had the greatest number of total roots. The latter had a greater

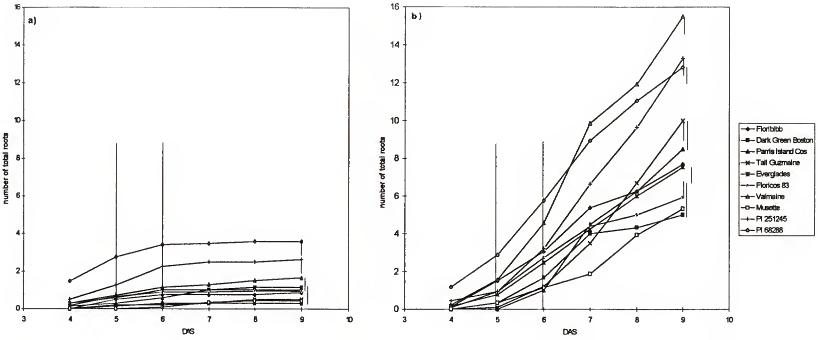

Figure 3-3. Number of total roots in lettuce seedlings grown from 4 to 9 DAS without (a) and with sucrose (b) in the medium. (Vertical lines at 5 and 6 DAS indicate separation of the phases of growth. Bars at 9 DAS indicate standard errors).

number of total roots than did the PI lines, mainly because 'Valmaine' produced relatively more basal roots. Thus, the presence of sucrose in the growing medium enhanced initiation of both lateral and basal roots, however, the response varied with genotype. All genotypes formed less basal roots than lateral roots at any point in time.

An interaction between genotype, sucrose and time affected lettuce taproot length (Table 3-2). 'Everglades' taproots were shorter than the other genotypes (Figures 3-4a and 3-4b). 'Everglades' is a butterhead lettuce selected for planting in Florida during late fall and winter since it is susceptible to bolting above 32°C (Guzman et al., 1992). Although 'Everglades' is a descendent of 'Dark Green Boston', their responses differed in terms of taproot elongation. 'Dark Green Boston' produced a longer taproot than 'Everglades', with and without sucrose in the medium, and it produced fewer lateral and basal roots. Taproots in both cultivars were longer when seedlings were grown in sucrose-enriched medium (Figure 3-4b).

Different cultivar responses to sucrose-enriched medium, including root branching and taproot growth, may be related to the genetic background of the material. Since species with longer and deeply penetrating roots, have been

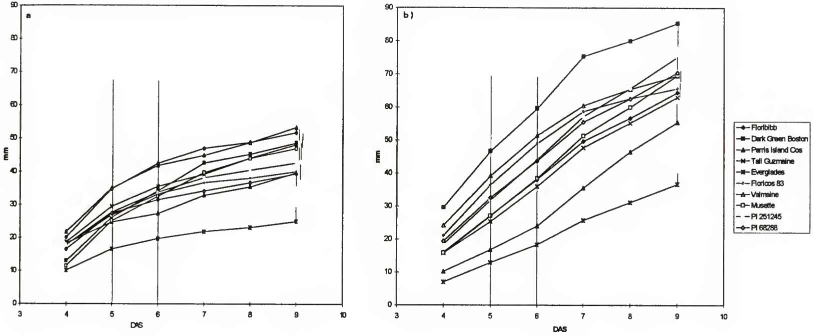

Figure 3-4. Taproot length of lettuce seedlings grown from 4 to 9 DAS without (a) and with (b) sucrose in the medium. (Vertical lines at 5 and 6 DAS indicate separation of the phases of growth. Bars at 9 DAS indicate standard errors).

demonstrated to be drought tolerant (Zobel, 1996), the root elongation capacity of 'Dark Green Boston' could favor this cultivar in arid soils. Conversely, the root growth habit of 'Everglades' with a shorter taproot and more basal and total root numbers, indicates that this cultivar would exploit shallow soils.

Analyses of Growth Phases

Environmental effects on plant growth can differ depending on the stage of root system and plant development. A capacity for rapid proliferation of roots early in plant growth could facilitate more efficient utilization of water and minerals, as well as enhancing tolerance to related stresses. Genotypes could be selected that exhibit such pattern of development during early growth, and from these, others which later shift allocation of photosynthetic resources to harvestable plant parts (lettuce leaves in this instance). In addition, root responses to enhanced supply of carbohydrate at various stages of development could help define the extent to which limitations in the overall process of root-shoot partitioning are external (environmental) or internal (genetic and/or developmental). Possibilities exist for successful manipulation of root-shoot partitioning by carefully-timed applications of exogenous sucrose and/or elevated CO_2 during key points in early seedling development.

During the early phase of growth (4 to 5 DAS), Phase 1, there was no significant interaction between genotype and sucrose for number of lateral, basal and total roots or for taproot length (Table 3-3). However, the presence of sucrose in the medium increased basal root number in Phase 1 (Table 3-4). Genotype affected the initial lateral and total root number, but not the basal root number or taproot length. Lateral root number increased during growth Phase 1 an average of 1.2 in 'PI 68288', more than any of the other genotypes (Table 3-5). Lateral root number increased an average of 0.4 in 'PI 251245', similarly in 'Floricos 83' and 'Valmaine'. Total root numbers increased during Phase 1 an average of 1.5 in 'PI 68288', more than any other genotype.

During growth Phase 2 (5 to 6 DAS), genotype continued to influence the number of lateral roots produced, wherein 'PI 68288' had the greatest number of lateral roots (1.6) (Table 3-5). In Phase 2, genotype did not affect basal and total root number, or taproot length (Table 3-3). Sucrose added to the medium increased lateral (Table 3-6), basal (Table 3-4) and total (Table 3-7) root numbers, as well as and taproot length (Table 3-8).

Table 3-3. P values of the different segments of trend analysis of lateral, basal and total number of roots and taproot length of lettuce seedlings DAS 4 through 9. (Phase 1 = 4 to 5 DAS; Phase 2 = 5 to 6 DAS; Phase 3 = 6 to 9 DAS, slope).

	Lateral Roots			Basal Roots		
Treatments	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
Genotype	0.0041	0.0375	0.0351	0.3476	0.1917	0.0948
Sucrose	0.5946	0.0001	0.0001	0.0170	0.0079	0.0001
Sucrose * Genotype	0.9836	0.5909	0.0656	0.3154	0.6427	0.0001
	Total Roots			Taproot Length		
	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
Genotype	0.0052	0.0961	0.0132	0.3062	0.2988	0.1741
Sucrose	0.1027	0.0001	0.0001	0.2574	0.0008	0.0001
Sucrose * Genotype	0.6565	0.6732	0.0289	0.1558	0.9830	0.3431

Table 3-4. Least Squares Means (LSM) and Standard Errors (SE) for the sucrose effect on basal root number of lettuce seedlings during Phase 1 and 2 of growth (Main effect significant at the ANOVA). (Phase 1 = 4 to 5 DAS; Phase 2 = 5 to 6 DAS).

	Phase 1		Phase 2	
Sucrose ($g\ l^{-1}$)	LSM	SE	LSM	SE
0	0.1	0.1	0.1	0.1
30	0.3	0.1	0.4	0.1

Table 3-5. Least Squares Means (LSM) and Standard Errors (SE) for the 10 genotypes of lettuce in lateral root number during the three phases of growth and in total root number during Phase 1 of growth (Main effect significant at the ANOVA). (Phase 1 = 4 to 5 DAS; Phase 2 = 5 to 6 DAS; Phase 3 = 6 to 9 DAS, slope).

	Lateral Roots						Total Roots	
	Phase 1		Phase 2		Phase 3		Phase 1	
Genotype	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Floribibb	0.6	0.2	0.7	0.3	0.3	0.2	1.0	0.3
Dark Green Boston	0.2	0.3	0.7	0.4	0.2	0.2	0.3	0.4
Parris Island Cos	0.1	0.3	0.6	0.4	0.4	0.2	0.1	0.3
Tall Guzmaine	0.1	0.2	0.3	0.3	0.5	0.2	0.2	0.3
Everglades	0.2	0.2	0.8	0.3	0.4	0.2	0.5	0.3
Floricos 83	0.4	0.2	0.7	0.2	0.2	0.2	0.7	0.2
Valmaine	0.4	0.2	1.1	0.2	0.7	0.2	0.9	0.2
Musette	0.1	0.2	0.3	0.2	0.3	0.2	0.2	0.2
PI 251245	0.4	0.2	1.0	0.3	0.8	0.2	0.6	0.3
PI 68288	1.2	0.2	1.6	0.2	0.5	0.2	1.5	0.2

Table 3-6. Least Squares Means (LSM) and Standard Errors (SE) for the sucrose effect on lateral root number of lettuce seedlings during Phase 2 and 3 of growth (Main effect significant at the ANOVA). (Phase 2 = 5 to 6 DAS; Phase 3 = 6 to 9 DAS, slope).

	Phase 2		Phase 3	
Sucrose ($g\ l^{-1}$)	LSM	SE	LSM	SE
0	0.3	0.1	0.0	0.1
30	1.3	0.1	0.8	0.1

Table 3-7. Least Squares Means (LSM) and Standard Errors (SE) for the sucrose effect on total root number of lettuce seedlings during Phase 2 (Main effect significant at the ANOVA). (Phase 2 = 5 to 6 DAS).

	Phase 2	
Sucrose ($g\ l^{-1}$)	LSM	SE
0	0.4	0.2
30	1.7	0.2

Table 3-8. Least Squares Means (LSM) and Standard Errors (SE) for the sucrose effect on taproot length of lettuce seedlings during Phase 2 and 3 of growth (Main effect significant at the ANOVA). (Phase 2 = 5 to 6 DAS; Phase 3 = 6 to 9 DAS, slope).

	Phase 2		Phase 3	
Sucrose ($g\ l^{-1}$)	LSM	SE	LSM	SE
0	6.0	0.8	1.6	0.5
30	10.6	0.7	4.2	0.5

Analysis of growth Phase 3 (6 to 9 DAS), indicated that the linear trend in growth of lateral root number (slope) was increased by both genotype and sucrose (Table 3-3). However, there was no interaction effect on lateral root number, and sucrose increased lateral root formation (Table 3-6). 'PI 251245' and 'Valmaine' formed more lateral roots in Phase 3 than 'Floricos 83' and 'Dark Green Boston' (Table 3-5). The greater final number of lateral roots of PI lines and 'Valmaine' 9 DAS compared to 'Tall Guzmaine' was due to an increase in lateral root formation during Phase 2.

An interaction between sucrose and genotype was also evident during Phase 3 for basal and total root number (Table 3-3). Although sucrose did not significantly affect basal and total root numbers in 'Dark Green Boston' between 6 to 9 DAS, basal and total root numbers were increased in all other genotypes examined (Table 3-9). In addition, sucrose-fed seedlings of PI lines and 'Valmaine' produced more total roots than the other genotypes. Basal root growth in response to added sucrose was also greater in 'Valmaine' than any other genotype and increased by an average of 0.5 basal root per day.

As during earlier phases of growth, genotype did not differ in taproot length during Phase 3 (Table 3-3). Sucrose, however, enhanced taproot elongation (Table 3-8).

Table 3-9. Least Squares Means (LSM) of beta values and Standard Errors (SE) for significant interaction between genotype and sucrose treatments during Phase 3. Basal and total root number of lettuce seedlings.

		Basal Roots		Total Roots	
Genotype	Sucrose (g l^{-1})	LSM	SE	LSM	SE
Floribibb	0	0.0	0.06	0.0	0.24
Floribibb	30	0.1	0.04	0.7	0.19
Dark Green Boston	0	0.0	0.06	-0.1	0.25
Dark Green Boston	30	0.1	0.09	0.5	0.35
Parris Island Cos	0	0.0	0.06	-0.0	0.26
Parris Island Cos	30	0.3	0.08	1.3	0.31
Tall Guzmaine	0	0.0	0.05	0.0	0.22
Tall Guzmaine	30	0.4	0.05	1.4	0.22
Everglades	0	0.0	0.06	0.0	0.25
Everglades	30	0.1	0.05	0.9	0.20
Floricos 83	0	0.0	0.04	0.0	0.19
Floricos 83	30	0.2	0.04	0.5	0.19
Valmaine	0	0.0	0.05	0.1	0.20
Valmaine	30	0.5	0.05	1.8	0.20
Musette	0	0.0	0.05	0.0	0.21
Musette	30	0.2	0.04	0.7	0.19
PI 251245	0	0.0	0.06	0.0	0.24
PI 251245	30	0.1	0.05	1.7	0.20
PI 68288	0	0.0	0.04	0.0	0.19
PI 68288	30	0.2	0.04	1.2	0.19

In conclusion, external applications of sucrose enhanced root growth mainly during Phase 2 and 3 of plant growth. This finding suggests that during the very early growth of lettuce (less than 5 DAS), seedlings are only utilizing food reserves provided in the seeds and are initially unable to use exogenous sucrose. When compared to other genotypes, the greater capacity of wild type lettuce seedlings to partition additional resources (sucrose) to root growth might suggest that the genetic selection by the breeders to improve shoot quality has occurred at the expense of the root system.

Plant Biomass 9 Days After Seeding

Sucrose significantly increased shoot and root dry mass 9 DAS and the extent of this effect varied with genotype (Table 3-10). Shoot dry mass increased in all genotypes when sucrose was added in the medium (Figure 3-7). This occurred to a greater degree in 'Valmaine', 'PI 251245' and 'PI 68288' than the other genotypes.

The greatest growth response to sucrose was evident in root dry mass (Figure 3-8). All genotypes were affected by sucrose-enriched medium, but partitioning to root dry mass was greatest in 'Valmaine' and 'PI 251245'. Root dry mass of these two genotypes increased fourfold when sucrose was

Table 3-10. Results of Analyses of Variance of lettuce biomass 9 DAS.

		Dry Mass		
		Shoot Mass	Root Mass	Root:Shoot Ratio
Treatments	DF	F Test	F Test	F Test
Genotype	9	0.0078	0.0001	0.0006
Sucrose	1	0.0001	0.0001	0.0001
Genotype * Sucrose	9	0.0448	0.0054	0.3966

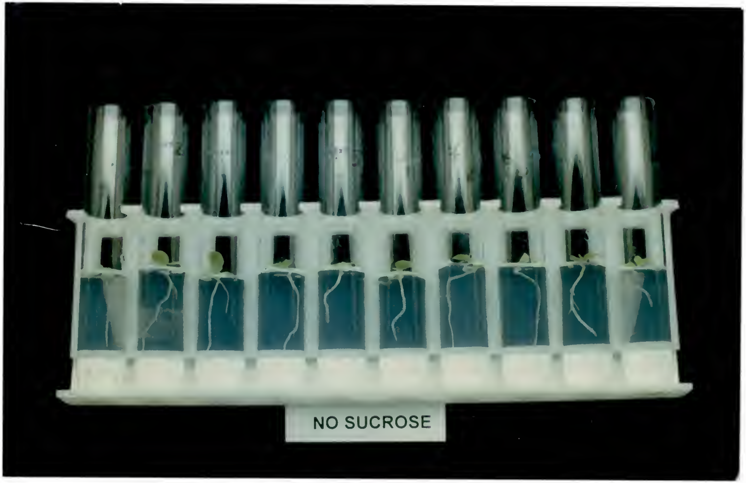

Figure 3-5. Lettuce seedling 9 DAS grown without sucrose in the medium.

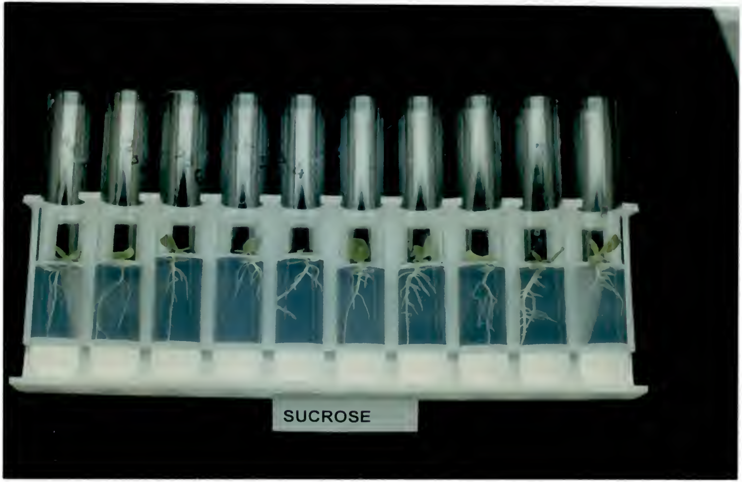

Figure 3-6. Lettuce seedling 9 DAS grown with sucrose in the medium.

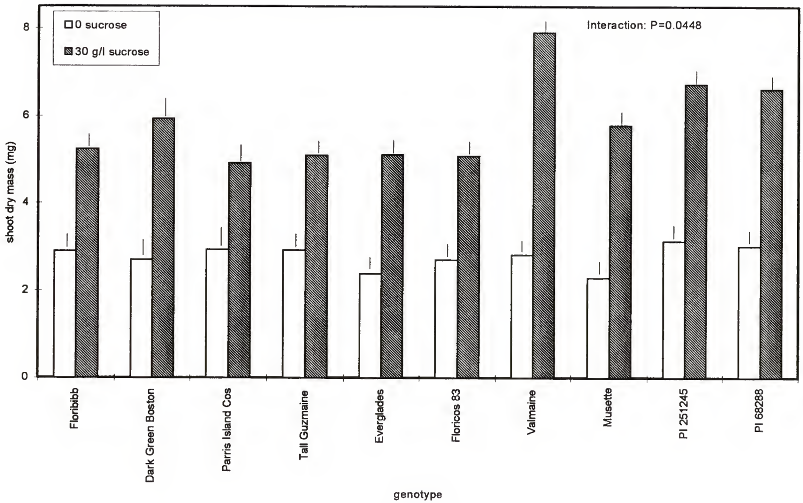

Figure 3-7. Shoot dry mass of lettuce seedlings 9 DAS (bars indicate standard errors).

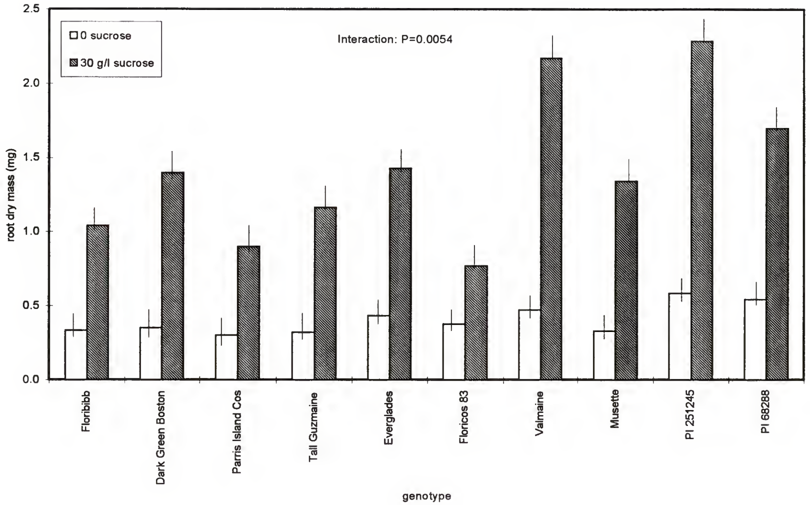

Figure 3-8. Root dry mass of lettuce seedlings 9 DAS (bars indicate standard errors).

added to the medium. Root biomass of 'Musette' and 'Tall Guzmaine' also increased fourfold when sucrose was added to the medium. The increase in root dry biomass could be attributed to a sucrose enhancement of root branch formation for 'Valmaine', 'PI 251245' and 'Tall Guzmaine'. In the case of 'Musette' sucrose-fed seedlings, root dry biomass increase could be attributed to a sucrose enhancement of root branch formation and of root elongation (Figures 3-3 and 3-4).

Root to shoot dry mass ratio varied among genotypes (Table 3-11) and was greatest in 'PI 251245'. Regardless of genotype, root to shoot dry mass ratio was enhanced by sucrose within 9 DAS (no interaction occurred) (Tables 3-10 and 3-12). Average root to shoot ratio increased from 0.15 to 0.24 when sucrose was made available to the 10 genotypes (Table 3-11).

Some developmental processes can be accelerated by the availability of exogenous sucrose (Kovtun and Daie, 1995). This does not appear to explain the shift in partitioning observed in the present work, however, because seedling growth rates were not simply increased. Partitioning of assimilates between shoot and root was altered, as indicated by data on increased root to shoot dry mass.

Table 3-11. Least Squares Means (LSM) and Standard Errors (SE) for differences between the 10 genotypes of lettuce root:shoot dry mass 9 DAS.

	Root:Shoot	
Genotype	LSM	SE
Floribibb	0.17	.02
Dark Green Boston	0.18	.03
Parris Island Cos	0.15	.03
Tall Guzmaine	0.17	.02
Everglades	0.23	.02
Floricos 83	0.14	.02
Valmaine	0.22	.02
Musette	0.19	.02
PI 251245	0.26	.02
PI 68288	0.22	.02

Table 3-12. Differences between the lettuce grown with or without sucrose in the medium on root:shoot dry mass ratio 9 DAS.

Sucrose ($g\ l^{-1}$)	Root:Shoot Dry Mass Mean
0	0.15
30	0.24
F Test	0.0001

Furthermore, root architecture was altered by sucrose feeding occurred. In 'PI 251245', for instance, exogenously applied sucrose led to a greater increase in the number of lateral roots (which rose from 1.8 to 11.3), than basal roots (which rose from 0.8 to 2.0) within 9 DAS. In 'Musette', sucrose application again increased lateral root number to a greater degree than basal roots (0.4 to 11.3 vs 0.0 to 1.4). However, in 'Dark Green Boston' no sucrose effect was observed in basal root formation. The enhancement of root growth by sucrose was, therefore, more pronounced on formation of lateral than basal roots and varied by genotype.

Collectively, the data from the present study suggest that exogenously supplied sucrose increased growth of lettuce seedlings, affected the partitioning of assimilates between root and shoot, and differentially increased the formation of primary root branches. This differential response of the root architecture to sucrose indicates it may play a regulatory role in root differentiation and whole plant physiology.

Wild species may have adapted to stressful environments at least partially by producing vigorous root systems. 'PI 251245' had a greater root to shoot dry biomass 9 DAS than the selected cultivars, confirming the greater capacity of wild lettuce to partition resources to root development. Moreover, biomass increase was in part due to additional root branch formation. Many lettuce cultivars have been selected under favorable growing conditions wherein root structure became less important to the plant as well the breeder and thus, vigorous root development may have been overlooked.

According to Zobel (1996), much of the recent emphasis on genotypic variation focused on a need for greater drought, salt, acidic soil resistance, or nutrient acquisition. Therefore, an extensive breeding effort is required to improve morphological and developmental characteristics of root systems. The present study indicated that genetic variability for rooting characteristics is present in lettuce, both cultivated and wild genotypes. Incorporating specific rooting patterns into new cultivars would be an effective mean of genetically improving new lettuce biotypes. Successful breeding programs for root characteristics have been implemented for edible root crops, such as carrot (*Daucus carota* L.) (Freeman and Simon, 1983). Survival and adaptation of any species to stress also depends on the root system. Nondestructive and observational methods of root characterization, such as accomplished in the present research, could be a valuable tool for breeding protocols, wherein a plant breeder could take into account root morphological characteristics in germplasm selection.

Summary

Wild species of lettuce may have adapted to stressful environments through production of more vigorous root systems. Current lettuce varieties have typically been usually selected under more favorable growth conditions in which root structure may have been less important to the plant as well the breeder. The wild progenitors of domestic lettuce are potential sources of valuable germplasm for enhancing the root system of this crop. Sugar supplies in plants provide carbon resources for growth and respiration, however, also regulate gene expression and carbohydrate partitioning within the plant. Root architecture of young lettuce seedlings grown for 9 days after seeding (DAS) was evaluated in ten genotypes [8 selected cultivars and 2 wild plant introduction (PI) lines] of lettuce (*Lactuca sativa* L.), either supplied or not with exogenous sucrose.

Taproot elongation, as well as numbers of lateral, basal and total roots, were affected by sucrose feeding and genotypes. Variability among genotype was more pronounced during the early phases of seedling growth (4 to 6 DAS), whereas the effect of exogenous sucrose feeding became more important in later root growth and development (5 to 9 DAS). The PI lines produced more lateral roots than the selected cultivars when exogenous sucrose was unavailable. Exogenous sucrose enhanced lateral root formation in all genotypes, but more in the PI lines, 'Valmaine' and 'Tall Guzmaine'. Sucrose increased the numbers of basal roots in all genotypes, except 'Dark Green Boston'. 'Valmaine' produced more basal roots than any other genotype when sucrose was added. 'Dark Green Boston', which differentiated few primary branches, produced the longest taproot within 4 to 9 DAS. The enhancement of root growth by sucrose was more pronounced on formation of lateral than basal roots and varied by genotype.

Sucrose increased final shoot and root dry mass (9 DAS). Shoot dry mass of all genotypes had increased when sucrose was added to the medium, however greatest increases were observed in 'Valmaine', 'PI 251245' and 'PI 68288'. Root to shoot dry mass ratio increased in lettuce seedlings when sucrose was added to the medium, and did so to the greatest extent in 'PI 251245'.

The present study indicated that exogenously supplied sucrose not only increased growth of lettuce seedlings, but also enhanced the partitioning of assimilates to roots vs shoots, and differentially increased the formation of lateral vs basal roots. This differential response of the root architecture to sucrose indicates it may play a regulatory role in root differentiation and whole plant physiology. Genetic variability for rooting characteristics was identified among cultivated as well as wild genotypes. These results indicate that appraisals of specific rooting patterns can be incorporated into development of new cultivars, and that this strategy could provide an effective mean of genetically selecting new superior lettuce biotypes.

CHAPTER 4
PHOTOSYNTHETIC WHOLE PLANT CULTURE (PWPC): A NOVEL GROWTH SYSTEM FOR STUDY OF ROOT GROWTH MORPHOLOGY AND ARCHITECTURE

Introduction

Improving transplant quality requires research related to plant development during the early stages of seedling growth. Root structure, morphology, or architecture may affect the size and growth rate of a shoot (Leskovar and Stoffella, 1995). Early root growth and development determine the optimum root system throughout the entire plant cycle, consequently affecting growth of the whole plant leading to optimization of yields. However, field methods to study root growth which enable repeated direct observations of undisrupted root growth have been extremely limited (Mackie-Dawson and Atkinson, 1991). As early as 1927, Weaver and Bruner (1927) lamented on the lack of studies on how a root system functions. During the 1980s an increase in interest of studying roots occurred, particularly related to the regulation of root growth and development (Feldman, 1984).

Studies on root morphology and architecture have attracted much research in the field of forestry and fruit crops, since these species have a long life span and have a high dependence on below-ground conditions, e.g. water supply, fertility, soil physical property, aeration. Research on vegetable crops has focused mainly on shoot growth, possibly since shoots are considered the main source for maximization of the edible part of the plant (even when the edible part of the plant is below ground, i.e. tubers, roots or stolons). Much is now known about early seedling shoot growth and development, unfortunately little is understood about root growth and development.

Many methods to study the root system have been developed in recent years. Mackie-Dawson and Atkinson (1991) reviewed many of these methods. There are 3 major groups of methods that can be used to assess root growth in the field. The methods comprise: 1) Excavation of the root system; 2) Direct observation; and 3) Indirect analyses. Methods from the first group require the removal of the root system from the soil, usually by washing, and can cause a major loss of root material when dealing with young seedlings. Early in development, much of the root system is very fragile, making full excavation and architectural analysis extremely difficult (Jackson, 1995). In studying root systems actively growing in soil, Jackson (1995) felt that a major difficulty was the extraction of fine roots and the measurement of root architectural variables.

Observation methods require a viewing surface that is inserted into the soil (Mackie-Dawson and Atkinson, 1991). The development of the root system *in situ* can then be seen through a window into the soil, allowing the same area of volume of soil and root system to be observed continuously. The major constraint in using these methods is that only a portion of the root can be observed. If a whole root viewing surface could be developed, observation methods could represent a major advance for study of the root system. Although hydroponic liquid culture allows direct observation of the entire root system, present growing conditions that prevent normal root hair development and may also alter other aspects of root morphology.

The extent and activity of the root system has been indirectly measured by relating the root function to supply of water and mineral nutrients to the plant (Mackie-Dawson and Atkinson, 1991). For example, a relationship between root length and maximum soil moisture depletion was observed in pasture species by Evans (1978). Consequently, variation in soil moisture with depth as determined by measurement of soil water potential has been used for estimating root activity through mathematical models. Similarly, the absorption of ^{32}P was found to be correlated with root length by Atkinson (1989). This relationship was studied using a combination of an observation window in a laboratory study and the injection of the radio-isotope in the soil. These indirect methods can be effective in understanding root architecture, however, only on a relatively gross scale.

Another approach to the study of early seedling growth is that of micropropagation in tissue culture, particularly where all external carbon is derived from CO_2 (Kozai, 1991a). In this type of autotrophic culture, growth and development are largely influenced by physical environmental factors which include light, CO_2, humidity, air flow speed, temperature, and O_2. However, use of photoautotrophic, micropropagation of whole seedlings is fairly new, moreover there is little research on the effects of the physical environment on photoautotrophic growth and development of whole seedlings.

Seedling growth *in vitro* does not require a supply of growth substances as does an explant or a cutting. In fact, the seed from which the seedling originates provides all the substances necessary for early growth. Seedlings can grow autotrophically if provided sufficient CO_2. When using airtight vessels for growing photoautotrophic seedlings, the CO_2 concentration was often measured to be as low as the CO_2 compensation point (less than 100 $\mu mol\ mol^{-1}$) during most of the photoperiod (Fujiwara et al., 1987; Kozai et al., 1992). Using loose caps or gas permeable film for capping the growth vessels resulted in CO_2 concentrations often lower than 200 µmol mol^{-1} (Kozai, 1991a) (which is lower than the atmospheric concentration of 350 µmol mol^{-1}). During the dark period, CO_2 concentration rose to 3000-9000 µmol mol^{-1}, but remained low (100-200 µmol mol^{-1}) for the entire light period.

Little research has been conducted *in vitro* using seeds and even fewer studies have been done using *in vitro* culture to study root architecture and morphology. Stoffella *et al.* (1988) used test tubes filled with gelled medium (gelrite) supplemented with mineral nutrients and sucrose as a carbon source (photo-mixotrophic growth), to characterize the early root morphology of bell pepper seedlings. Transparent gelled medium was tested in preliminary investigations as a medium to study lettuce root development (Nicola *et al.*, 1996). Nicola *et al.* (1996) and Nicola (Chapter 3) used test tubes filled with Phytagel gelled medium supplied with mineral nutrients and sucrose to grow lettuce seedlings and study root morphology and architecture (Photo-Mixotrophic Whole Plant Culture, PMWPC). Plants were grown 9 days in the tubes and then removed. The lack of recyclable air and depth for root extension did not permit further growth. The hypothesis that autotrophic plant growth was possible using this growing system was addressed with the attempt of removing the exogenous sucrose supply as a carbon source and the introduction and circulation of fresh air into the plant container to avoid the lowering of CO_2 concentrations during the light period.

A whole three-dimensional viewing surface for observation of root growth has not been developed. Once developed it could represent a major advance for study of the root system. The objective of the present study was to design, develop and evaluate a system to study seedling root growth, which would allow non-destructive root measurement such as root count and allow the direct observation and description of the root architecture of lettuce seedlings in an autotrophic plant culture system.

Materials and Methods

Description of the Photosynthetic Whole Plant Culture (PWPC): The System

Forty 1-l glass bottles (Pyrex, Corning, NY) (Figure 4-1) were used to accommodate a quantity of medium (650 ml), which would permit the growth of lettuce seedlings for up to 18 days. Bottles were externally covered with aluminum foil around the medium to block light from reaching the root

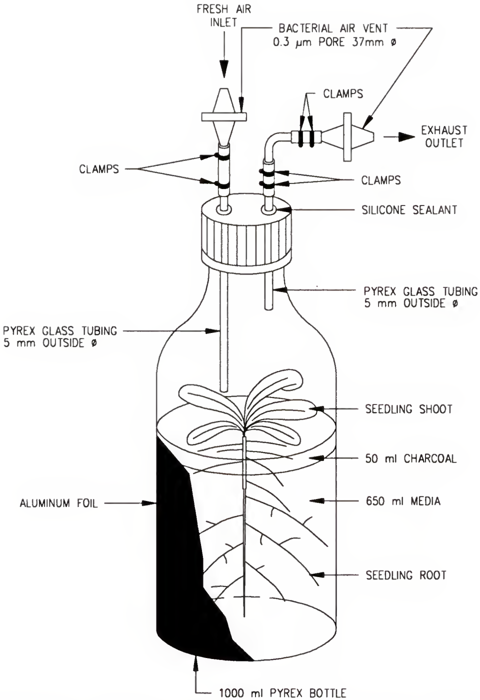

Figure 4-1. Diagram of a bottle and the parts added for lettuce seedling growth.

surface (Figure 4-1). The bottle cap was drilled with two holes to insert Pyrex glass tubing (5 mm outside diameter) allowing air flow to the plant (Figure 4-1). A tube was connected to the fresh air inlet with a bacterial vent (0.3 µm pore, GelmanSciences, Ann Arbor, MI); another tube was connected to the exhaust outlet with another bacterial vent (0.3 µm pore, GelmanSciences, Ann Arbor, MI). The holes in the bottle caps were sealed with silicone gel.

The air flowing into the bottle was filtered through a bacterial air vent (0.2 µm pore, GelmanSciences, Ann Arbor, MI) and humidified by passing the air into a flask containing a layer of sterilized-deionized water (Figure 4-2). Air flow was calibrated at 100 ml min^{-1} per bottle (Figure 4-3) prior to each experiment, then was monitored during the experiments by a control panel (Figure 4-4). A pump was placed before the flow calibrator to maintain constant pressure in the system. Air was preconditioned to 23°C and 75% RH in a controlled growth room, provided with an air dehumidification system prior to reaching the air flow calibrator (Figure 4-4). When air was introduced with CO_2, a gas mixing board was used to control CO_2 concentration in the bottles (Figure 4-4).

A bench (1219x914x152 LxWxH mm) was built to accommodate forty bottles (Figure 4-5). Light was provided

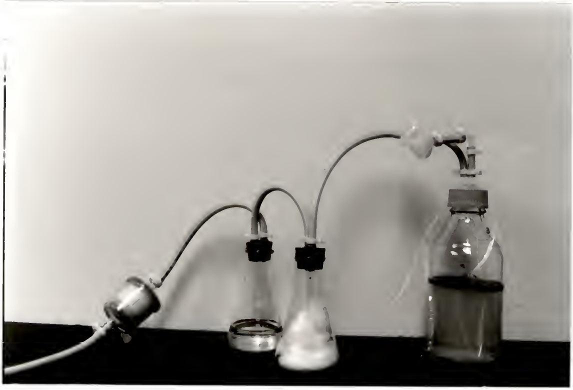

Figure 4-2. Bacterial Air Vent (0.2 µm pore) filtering the air prior re-humidification and prior to reaching the interior of the bottle.

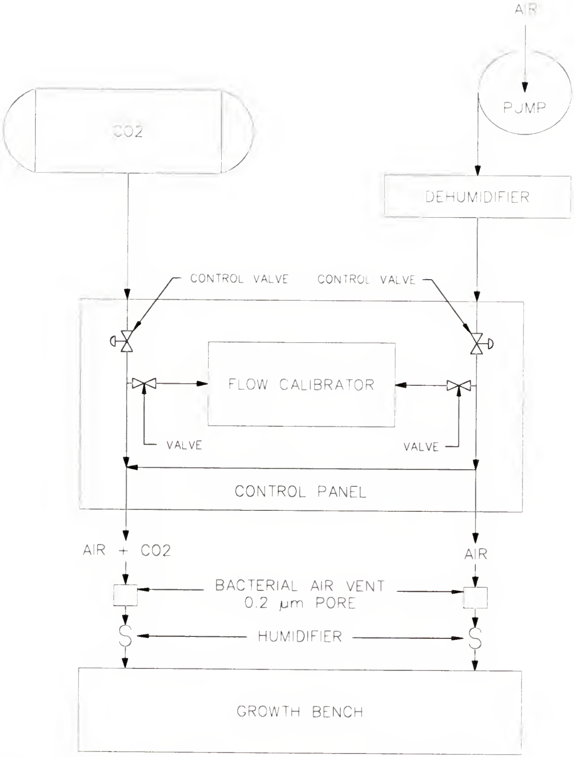

Figure 4-3. Scheme of the growth room.

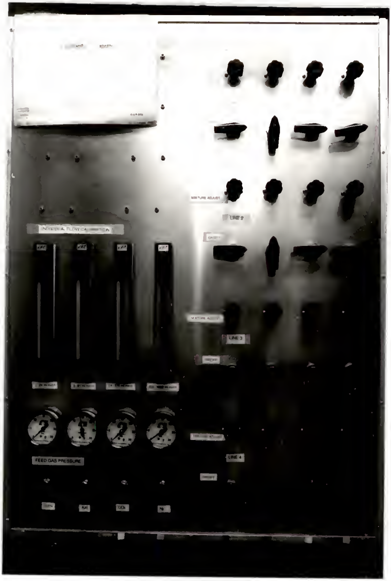

Figure 4-4. Picture of the control panel showing the knobs for air flow control (right), the anemometers for pressure control (left).

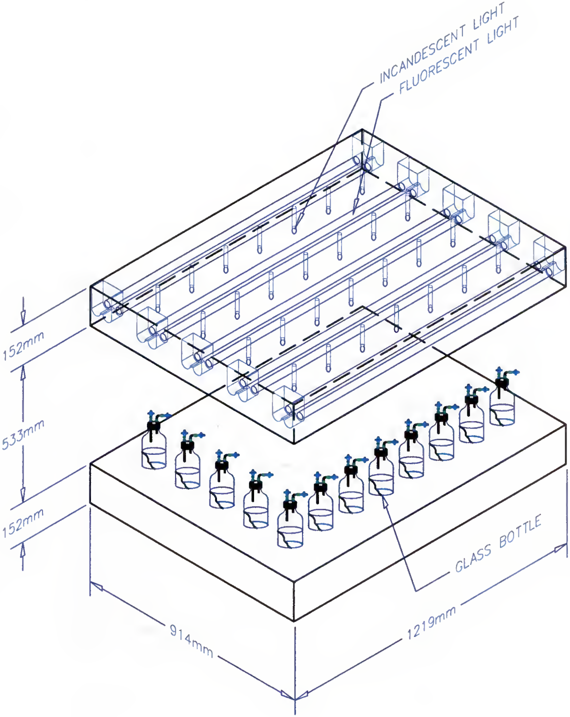

Figure 4-5. Scheme of the bench for plant growth.

with ten cool-white fluorescent tubes (Osram Sylvania Inc., Danvers, MA), each emitting 150 W light, and 10% of the total wattage (which was 1692 W) was provided by 48 incandescent bulbs (General Electric Co., Nela Park, Cleveland, OH), each emitting 4 W light, to supply the plants with far red light and a total light spectrum (Cathay and Campbell, 1980; Hart, 1988; Krizek and McFarlane, 1983; Murakami *et al.*, 1992; Smith, 1982) (Figure 4-5). As fluorescent lamps have low thermal infrared irradiation (Ikeda *et al.*, 1987), lamps were arranged 0.5 m from the base of the bench (Figure 4-5) to deliver 250 to 300 $\mu mol\ m^{-2}s^{-1}$ photosynthetic photon flux (PPF). The PPF was measured in each experiment with a quantum sensor (model LI-185; LI-COR, Lincoln, NE). The plants were grown under 16-hr light and 8-hr dark cycles. Temperature in the room was kept constant at 23°C to maintain an internal bottle temperature of 28/23°C ± 1°C (day/night).

Description of the Photosynthetic Whole Plant Culture System (PWPC): Plant Growth Medium

Basal nutrient medium contained inorganic salts according to Murashige and Skoog (1962) (Table 4-1). When sucrose was added to the medium, 30 g l^{-1} (90 mM) was used (Chapter 6). When nitrogen (N) was added to the medium to reach a total of 120 mM N, 60 mM N as NH_4NO_3 was used

Table 4-1. Composition of Murashige and Skoog (1962) medium for tissue culture

Major elements				Minor elements		
Salts	mg/l		mM	Salts	mg/l	µM
NH_4NO_3	1650	N	41.20	H_3BO_3	6.2	100
KNO_3	1900	N,K	18.80	$MnSO_4 \cdot 4H_2O$	22.3	100
$CaCl_2 \cdot 2H_2O$	440	Ca	3.00	$ZnSO_4 \cdot 4H_2O$	8.6	30.0
$MgSO_4 \cdot 7H_2O$	370	Mg,S	1.50	KI	0.83	5.0
KH_2PO_4	170	P,K	1.25	$Na_2MoO_4 \cdot 2H_2O$	0.25	1.0
Na_2EDTA	37.3	Na	0.20	$CuSO_4 \cdot 5H_2O$	0.025	0.1
$FeSO_4 \cdot 7H_2O$	27.8	Fe	0.10	$CoCL_2 \cdot 6H_2O$	0.025	0.1

(Chapter 7). Solution pH was adjusted to 6.5 with 1 N NaOH before autoclaving. Prior to autoclaving, 2.5 g l^{-1} of Phytagel (Sigma Chemical Co., St. Louis, MO) was added to the solution. The medium (650 ml) was poured into the bottle. After autoclaving for 35 min the medium was cooled to solidify at ambient temperature. A 50-ml layer of gel containing the same concentration of nutrients and Phytagel plus 3.5 g l^{-1} of activated charcoal (Grand Island Biological Company, Grand Island, NY) was added to the gelled surface under sterile conditions to reduce light in the root zone and avoid Fe-catalyzed Photooxidation of EDTA present in the substrate (Hangarter and Stasinopoulos, 1991). Since each bottle had a total volume of 1100 ml, 400 ml of air space remained above the medium.

Seeds of lettuce were surface-sterilized by soaking them in a solution of 2% NaOCl and 0.1% Tween 20 for 20 minutes. The seeds were then rinsed five times in sterilized-deionized water. All seeds were placed in a Petri dish with filter paper n.3 (Whatman International Ltd. Maidstone, England) moistened with sterilized-deionized water. The seeds were germinated for 24 hr at 20-22°C under continuous fluorescent light (Osram Sylvania Inc., Danvers, MA, 50 µmol $m^{-2}s^{-1}$ PPF). When root length reached 2 mm, one seedling was transferred into each bottle by placing the seed at the center top surface of the gelled medium. The seedling was carefully inserted into the medium leaving the upper hypocotyl above the gel surface. Forty glass bottles containing one seedling each were placed in the growth room on the bench as previously described. In all experiments plants were arranged in 8 lines of 5 plants each (Figure 4-5).

Description of the Photosynthetic Whole Plant Culture System (PWPC): Data Collection

The number of basal and lateral roots and the number of differentiated leaves were counted every 24 hr for 18 days following seeding, except in one experiment (13 days), by visual observation through the glass growth bottle. Total root number (lateral + basal roots) could be computed at any given point in time. Secondary branches emerging from the lateral roots were counted from the day the first branch root appeared until harvest, by direct observations through the glass growth bottle. At the end of the growing period, seedlings were gently removed from the bottles upon separating the gel in the bottle with a pair of forceps, and then they were washed manually under running water. At harvest, root and shoot dry weights were obtained after oven drying each seedling for 96 hr at 60°C. Taproot length was measured for each plant with a ruler with an approximation of 1 mm.

Data were analyzed using a SAS statistical system (SAS Institute Inc., 1989). Daily measurements were submitted to the analysis of variance of repeated measurements and the Greenhouse-Geisser adjusted P values were used (Greenhouse and Geisser, 1959) (Chapter 8). At harvest, data were also submitted to the analysis of variance.

Description of the Photosynthetic Whole Plant Culture System (PWPC): Plant Growth

A series of experiments was conducted to identify the efficiency of the growing system. These experiments were as follows:

1) Agar (Sigma chemical Co., St. Louis, MO) is generally used as a gelling agent in plant tissue culture (Ichimura and Oda, 1995). However, a study was conducted to determine the clarity of the solidifying media to be used for direct observations of the root growth, by comparing agar (7 g l^{-1}) and Phytagel (1.5, 2, 2.5 g l^{-1}) as a growth support medium.

2) It was also necessary to determine whether an initial 6.0 pH of the growing medium was altered by environmental conditions during 22 days of plant growth. Thirty-two bottles were filled as previously described.

Sixteen bottles contained seedlings grown in medium and 16 contained only the medium in order to determine the effect of plant growth on medium pH. Two levels of nitrogen (60 and 120 mM) and 2 concentrations of CO_2 in the air (350 µmol mol^{-1}, standard air and 2000 µmol mol^{-1}, CO_2-enriched air) were also examined. Final medium pH was measured at 4 depths: at the medium surface (0 mm) and at 20, 40 and 60 mm depth. The experimental design was a split-plot design with CO_2 as a main-plot treatment, N and plants as sub-plot treatments, 2 blocks with 2 plants each. Data of pH measures were submitted to the analysis of variance.

3) Since pH can be altered by autoclaving (Linsmaier and Skoog, 1965; Skirvin et al., 1986), tests were completed to predict the desired after-autoclaving pH of 6±1 for seedling growth. Six prior-to-autoclaving medium pH levels (Table 4-3) were examined in Murashige and Skoog medium without sucrose and 2.5 g l^{-1} Phytagel.

Results and Discussion

An *in vitro* culture system generally uses an agar-like medium, which upon solidification becomes translucent. However, gelled media (Phytagel) was more transparent upon solidifying (data not shown), thus making the root system easier to observe. Ichimura and Oda (1995) examined the effect of seven types of agar and other polysaccharides (cellulose, gelrite, pectin, sodium alginate and starch) on lettuce root growth. Agar stimulated more root growth than the other polysaccharides, implying that commercial agar contained root growth stimulation substances. Based on Ichimura and Oda (1995) and on our finding, Phytagel was chosen at the found optimal concentration of 2.5 g l^{-1}.

The medium pH at 0, 20, 40 and 60 mm of depth in the medium 22 DAS was not affected by CO_2 concentration in the air, by N level in the medium, nor by their interaction, regardless of the presence of a seedling in the medium (Table 4-2). Thus, CO_2 enrichment did not dissolve in the medium. The layer of charcoal most likely represented a barrier to CO_2 acidification of the medium. The presence of seedlings caused an acidification of the medium over time at any measured depth (Table 4-2). Plant roots have been reported to either increase (Liu, 1980; Marschner et al., 1986) or decrease rhizosphere pH (Grinsted et al., 1982; Shaller, 1987). The pH levels were lower closer to the surface (0 to 40 mm depth) where seedlings produced basal and lateral roots. Riley and Barker (1970) and Nye (1986) reported that pH changes are usually restricted to the rhizosphere. This would be confirmed by the present study. Any of the interactions with seedlings were not significant. Lettuce

Table 4-2. Changes in pH at 4 depths in the medium 18 DAS as influenced by 2 levels of N in the medium (60 and 120 mM), 2 concentrations of CO_2 in the air (350 and 2000 µmol mol^{-1}) and either absence or presence of seedlings in the medium.

Treatments			Depth			
CO_2 (µmol mol^{-1})	N (mM)	Seedlings	0 mm	20 mm	40 mm	60 mm
			pH values			
350			5.1	5.2	5.2	5.4
2000			4.9	4.8	4.8	5.0
Probability (F-test)			0.1748	0.1313	0.2037	0.1182
	60		5.0	5.0	5.0	5.2
	120		5.0	5.0	5.0	5.2
Probability (F-test)			0.9519	0.7102	0.8954	0.5969
		no	5.3	5.4	5.4	5.4
		yes	4.7	4.6	4.7	5.0
Probability (F-test)			0.0030	0.0001	0.0001	0.0001
350	60		5.1	5.2	5.2	5.3
350	120		5.1	5.2	5.2	5.4
2000	60		4.9	4.8	4.8	5.1
2000	120		4.9	4.7	4.8	5.0
Probability (F-test)			0.9587	0.2495	0.7840	0.1853
	60	no	5.4	5.3	5.3	5.4
	60	yes	4.6	4.6	4.7	5.0
	120	no	5.3	5.4	5.4	5.4
	120	yes	4.7	4.6	4.6	5.0
Probability (F-test)			0.7379	0.5315	0.1885	0.7491
350		no	5.5	5.6	5.6	5.6
350		yes	4.7	4.8	4.9	5.1
2000		no	5.1	5.1	5.1	5.3
2000		yes	4.6	4.4	4.5	4.8
Probability (F-test)			0.3990	0.7551	0.8206	0.9148
350	60	no	5.6	5.6	5.6	5.6
350	120	no	5.5	5.6	5.6	5.6
2000	60	no	5.2	5.1	5.1	5.3
2000	120	no	5.1	5.1	5.2	5.3
350	60	yes	4.7	4.8	4.9	5.1
350	120	yes	4.8	4.8	4.9	5.2
2000	60	yes	4.6	4.5	4.5	4.8
2000	120	yes	4.6	4.3	4.4	4.8
Probability (F-test)			0.9725	0.3195	0.2157	0.9559

seedlings can be grown in a wide range of pHs, from 4 to 8, with an optimum between 6 and 7 (Bianco, 1990). Consequently, a pH of 4.7, 22 DAS, was considered slightly low. Further tests were conducted.

Autoclaved media increased the original buffered pH when pH was lower than 5 (Table 4-3). When the initial pH was 5 or greater, autoclaving the medium decreased its pH. Low medium pH (below 5) prior to autoclaving prevented sufficient solidification. As Linsmaier and Skoog (1965) reported, a before-autoclaving pH of 6.5 resulted in an after autoclaving pH of 6.2. Thus, a medium pH of 6.5 was recommended for all subsequent experiments.

Lack of methods to study root growth which enable direct observation of the roots without their disruption generally limits study on root architecture. A whole three-dimensional viewing surface for observation of root growth has been developed. The present research has elucidated the methodology for design, development and evaluation a growing system which allowed non-destructive direct observation of seedling root growth. The growing system developed in this study enabled precise and accurate plant measurements, from radicle protrusion to plant harvest, under controlled environmental conditions. It could be a valuable tool to investigate root morphology and development in different

Table 4-3. Changes in pH of the Murashige and Skoog medium due to autoclaving.

Medium pH Before autoclaving	Medium pH After autoclaving
4.0	4.3
4.5	4.6
5.0	4.7
5.5	5.2
6.0	5.6
6.5	6.2

genotypes, to better study, select and breed new varieties based on their root architecture potential.

Summary

Early seedling growth and development of root systems determine root architecture throughout the entire plant life cycle, consequently affecting plant growth and subsequent yields. However, a three-dimensional viewing surface for observation of root growth has to date not been developed. The present research describes the methodology for design, construction and testing a growth system which permits non-destructive direct observation of root system growth. Pre-germinated lettuce seeds were placed on a Phytagel medium containing Murashige and Skoog salts in 1-l glass bottles. Air flow to the bottles was filtered, humidified and calibrated. A bench was constructed in a controlled temperature room and illuminated with 250 to 300 µmol $m^{-2}s^{-1}$ PPF. Internal bottle temperature was maintained at 28/23°C day/night by controlling the temperature within the room.

Phytagel at 2.5 g l^{-1} was more transparent upon solidification than Agar. The pH of the medium did not change over 22 days and was not altered by 2 levels of N (60 and 120 mM N) in the medium or 2 concentrations of CO_2 (350 and 2000 µmol mol^{-1} CO_2) in the air. Normal seedling growth over 22-day period caused an acidification of the medium. Autoclaving increased the medium pH when the original buffered pH was below than 5. When the initial medium pH was above 5, autoclaving decreased pH.

The described and evaluated seedling growing system was easily constructed, calibrated and monitored during seedling root growth studies. It enabled direct non-destructive plant observations and measurements, from radicle protrusion to plant harvest, under controlled environmental conditions. It may be a valuable tool to investigate root morphology and development and as a selection method to breed new varieties based on their root architecture potential.

CHAPTER 5

ROOT MORPHOLOGY AND ARCHITECTURE IN YOUNG LETTUCE SEEDLINGS GROWING IN A PHOTOSYNTHETIC WHOLE PLANT CULTURE (PWPC) SYSTEM

Introduction

The root is the lower portion of a plant axis which generally develops below the soil surface. A root system consists of main axes and their branches: vertical axes or taproots, first-order branches and higher-order branches. The apical meristem of branching roots develops deep within the inner tissues (at the periphery of the vascular cylinder, Esau, 1977) in contrast to the buds of the shoot, which develop from outer tissues (outside the vascular cylinder, Fahn, 1982). Therefore, branching of roots is endogenous and that of stems exogenous (Esau, 1977; Fahn, 1982).

The production of a primary root system, i.e. the primary branching from the radicle, is an important phenomenon in growth and survival of a plant (MacIsaac et al., 1989). A primary root system increases the surface area available for the uptake of water and mineral elements. In addition, with its architecture, a primary root system gives support of the shoot to the substrate or soil.

Root architecture can be very important in plant productivity. Many of the soil's resources are unevenly distributed, or are subjected to localized depletion by the roots, so that the spatial distribution of the root system can determine the capacity of a plant to exploit those resources and to respond to localized availability of them (Lynch, 1995). Patches of localized nutrients in the soil, such as P, may be available to the root system only if roots have the potential to reach the patches. Gradients in temperature, O_2 and CO_2 content, pH, and water availability, commonly occur in soils. Soil gradients, such as soils richer in nutrients at the surface or soils which are more subject to drought and/or temperature extremes, may also occur. Seasonal gradients in soils change the mobility of mineral nutrients such as N. Warmer temperatures improve uptake of NO_3 by the root, whereas cooler temperatures favor uptake of NH_4 (Mengel and Kirkby, 1982). Exploitation by the root system of soil resources may consume more than 50% of the available photosynthate in mature plants (Fogel, 1985).

Generally, seedling root morphology differs between monocotyledonous and dicotyledonous species (Fahn, 1982; Leskovar and Stoffella, 1995; Sutton and Tinus, 1983; Zobel, 1975, 1986). Roots of dicotyledonous species are generally classified into 4 types (Klepper and Rickman, 1991;

Stoffella et al., 1988; Zobel, 1975, 1986, 1996): radicle, adventitious, lateral and basal. The radicle will form the taproot (primary root), adventitious roots are initiated from the hypocotyl, lateral roots from the taproot, and basal roots from the "lower hypocotyl and upper tap root," according to Zobel (1986) or from the "basal part of the hypocotyl or shoot-root transition zone when pericycle cells do not originate in this area," according to Leskovar and Stoffella (1995).

Zobel (1986) indicated that initiation of basal roots is under different genetic control from initiation of lateral and adventitious roots. A double homozygote from a lateralless tomato mutant (recessive mutant called diageotropica, *dgt*) and an adventitiousless tomato mutant (recessive mutant called rosette, *ro*) originated roots in the hypocotyl and upper portion of the taproot. Assuming only 3 types of roots, the double homozygote should have had only a taproot. Since these roots were genetically not lateral, nor were they adventitious, Zobel (1975) classified these roots as 'basal' roots.

Adventitious roots cannot be confounded with lateral roots for two main reasons. First, adventitious roots originate from the stem, while lateral roots originate from the taproot. Second, the adventitious roots originate from tissues other than the pericycle, while the lateral roots originate from the pericycle. Conversely, basal roots are not clearly classified with respect to their point of origin. Zobel (1986) stated that he demonstrated that basal roots originate from the pericycle of the lower hypocotyl and upper taproot. Consequently, basal roots were not adventitious in anatomical origin, nor lateral or adventitious in genetic control of their initiation. Leskovar and Stoffella (1995) indicated that basal roots originate from a transition zone between the shoot and root.

Eshel and Waisel (1996) reported that basal roots are less sensitive to gravity than lateral roots, and therefore can extend the root system horizontally. The authors attributed the major function of the basal roots as exploiting the most fertile portions of agricultural soils, more efficiently than lateral roots. Bole (1977) reported that basal roots of rape (*Brassica campestris* L.) absorbed nutrients (P) more efficiently than lateral roots.

Adventitious roots in lettuce seedlings have not been reported. Whether a distinction between basal and lateral root development in lettuce seedlings exists has not been determined. Once this distinction has been established in lettuce, another question that might be asked is whether basal root emergence occurs prior to or after lateral root emergence.

In bell pepper seedlings, basal roots emerged prior to lateral roots (Stoffella et al., 1988) and only after full cotyledon expansion. In tomato seedlings, lateral roots emerged prior to basal roots (Aung, 1982). Weinhold (1967) described basal roots as arising acropetally (toward the shoot apex) from the radicle, while lateral roots arose basipetally (toward the radicle apex). Charlton (1996) did not distinguish between lateral and basal roots, but he defined lateral roots as "roots derived from lateral endogenous primordia formed in preexisting roots". The author stated that lateral roots appeared at a relatively constant distance behind the tip of a growing root, that lateral roots initiated in rows or ranks, and that within each rank they appeared to initiate and emerge in acropetal sequence under normal conditions. Charlton (1996) reported that between the basipetally emerged lateral roots, additional laterals may arise for a long period in roots with secondary growth. Esau (1965) termed these lateral roots adventitious.

Lettuce root systems have been described as early as 1927 by Weaver and Bruner (1927). The authors described the "common lettuce of the garden (*Lactuca sativa crispa*) as a tall, leafy, annual herb with a milky juice" (p.320). The term "milky juice" can easily be related to the probable origin of the Latin name *Lactuca*, which appears to derive from the word *lactucus*, i.e., "rich in milk" (Bianco, 1990, p.270).

To describe root system development, Weaver and Bruner (1927) planted 'Early Prize Head' lettuce seeds in soil. Early root growth has been described to take place rapidly, with lateral branches appearing on the first 25 to 40 mm of the taproot, in conditions very favorable for growth, 6 days after cotyledons unfolded. These first branches were described as arising horizontally and very near to the soil surface. Thirty-eight days after seeding, at the first examination in the field, the plants were 75 mm tall, the taproots were 5 mm in thickness near the soil surface and gradually tapered to 1 mm towards the root tip. Taproots were course and grew almost vertically downward for 500-700 mm in depth and unbranched in the last 150 to 200 mm. Branches arose on two opposite sides of the taproot, mostly in the first 300 mm of soil. Sixteen roots were counted arising from 25 to 50 mm below the surface, followed by 7 to 14 roots arising every 25 mm of taproot length for about 150 mm. After that, fewer, short and unbranched primary branches were found closer to the taproot tip. The authors reported that in the surface 150 mm of soil, many of the roots ended within 5 mm of the taproot, whereas others extended horizontally 100 to 200 mm, and a few had a length of 400 to 500 mm. Branches of the third order were noted on the oldest roots, sometimes reaching a length of 17 mm, while below 100 mm, the lateral roots were unbranched.

Jackson (1995) hypothesized that *Lactuca sativa* (cultivated type) and *Lactuca serriola* (wild type) differed in root architecture, on the basis of timing and distribution of water and nutrients in the soil, and that inadvertent selection has occurred for root characteristics in *L. sativa,* that would contribute to rapid growth and shoot uniformity under cultivation practices that utilize large amounts of water and N. The author conducted a series of experiments addressing the genetic and management controls on nutrient cycling in agricultural systems. He grew seedlings of *Lactuca sativa* cv. Salinas and *Lactuca serriola* for 5 to 6 weeks in a greenhouse. The studies were conducted using either pots or cylinders filled with coarse-textured soil. Root biomass allocation was very similar at 4 to 6 weeks after transplanting, but root architecture differed. In the top zone along the taproot (0 to 50 mm), cultivated lettuce tended to produce more lateral roots (probably this type of root is what other authors call basal root), a greater total root length, and more external links [(segments that originate at a branch point and end in a meristem, referred to as *magnitude* by Fitter (1996)] than the wild lettuce. Conversely, from the 50 to the 550 mm zone of the taproot, wild lettuce produced more laterals than *L. sativa*. Jackson (1995) concluded that inadvertent selection may have occurred in the breeding of cultivated lettuce varieties. These selections occurred for increased root growth in the surface zone where water and fertilizers were applied and for greater plasticity in construction of root segments, which may maximize the efficiency of exploitation of soil moisture and nutrients.

Basal roots have been reported to be produced by mungbean (*Vigna radiata* L.) (Leskovar and Stoffella, 1995), bell pepper (*Capsicum annuum* L.) (Stoffella et al., 1988), tomato (*Lycopersicon esculentum* Mill.) (Zobel, 1975) and beans (*Phaseolus vulgaris* L.) (Stoffella *et al.*, 1979). To our knowledge, however, the literature does not report any description of root architecture in lettuce, besides previously described. Understanding whether differentiation between lateral and basal root formation occurs in lettuce may be valuable to improve growth rates of lettuce seedlings. Detailed information on the sequence of events occurring in root branch formation of seedlings grown in a controlled environment could be valuable to further determine the root development of field-grown plants. Consequently, the objective of the present study was to describe the root morphology, growth and architecture of young lettuce seedlings grown in a Photosynthetic Whole Plant Culture (PWPC) system.

Materials and Methods

Gelled medium 'Phytagel' (Sigma Chemical Co., St. Louis, MO) (2 g l^{-1}) was used as a substrate for growing and evaluating root morphology and development of lettuce seedlings from radicle protrusion to 18 days after seeding (DAS). The basal nutrient medium contained inorganic salts according to Murashige and Skoog (1962) (Table 5-1). The solution pH was adjusted to 6.5 with 1 N NaOH prior to autoclaving. Forty 1-l glass bottles (Pyrex, Corning, NY) were prepared by pouring 650 ml of medium and autoclaving the bottles for 35 min at 130° C at 1.1 kg cm^{-2} pressure (15 psi). After autoclaving, the medium was cooled to solidify at ambient temperature. A 50-ml layer of gel containing the same concentration of nutrients and Phytagel plus 3 g l^{-1} of activated charcoal (Grand Island Biological Company, Grand Island, NY) was added to the gelled surface to reduce light in the root zone and avoid Fe-catalyzed photooxidation of

Table 5-1. Composition of Murashige and Skoog (1962) medium for tissue culture.

Major elements				Minor elements		
Salts	mg/l		mM	Salts	mg/l	µM
NH_4NO_3	1650	N	41.20	H_3BO_3	6.2	100
KNO_3	1900	N,K	18.80	$MnSO_4 \cdot 4H_2O$	22.3	100
$CaCl_2 \cdot 2H_2O$	440	Ca	3.00	$ZnSO_4 \cdot 4H_2O$	8.6	30.0
$MgSO_4 \cdot 7H_2O$	370	Mg,S	1.50	KI	0.83	5.0
KH_2PO_4	170	P,K	1.25	$Na_2MoO_4 \cdot 2H_2O$	0.25	1.0
Na_2EDTA	37.3	Na	0.20	$CuSO_4 \cdot 5H_2O$	0.025	0.1
$FeSO_4 \cdot 7H_2O$	27.8	Fe	0.10	$CoCL_2 \cdot 6H_2O$	0.025	0.1

EDTA present in the substrate (Hangarter and Stasinopoulos, 1991).

Seeds of lettuce (*Lactuca sativa* L.) cv. Valmaine were surface-sterilized by soaking them in a solution of 2% NaOCl and 0.1% Tween 20 for 20 minutes. The seeds were then rinsed five times in sterilized-deionized water, placed in a Petri dish with filter paper n.3 (Whatman International Ltd. Maidstone, England), and wetted with sterilized-deionized water. The seeds were germinated for 24 hr at 20-22°C under continuous fluorescent light (Osram Sylvania Inc., Danvers, MA, 50 μmol $m^{-2}s^{-1}$ PPF). When radicle length reached 2 mm, one seedling was transferred into each bottle by placing it on the center top surface of the gelled medium. The seedling was carefully inserted into the medium leaving the upper hypocotyl of the seedling above the gel surface. Glass bottles were externally covered with aluminum foil around the medium to block light from reaching the root surface. Bottles were placed in the growth room on the bench as previously described (Chapter 4). The total PPF was 270 μmol $m^{-2}s^{-1}$, obtained by a combination of ten cool-white fluorescent tubes (Osram Sylvania Inc., Danvers, MA), each emitting 150 W light, and 48 incandescent bulbs (General Electric Co., Nela Park, Cleveland, OH), each emitting 4 W light. The lamps were arranged at 0.5 m from the base of the bench, and the photoperiod was 16 hr. Temperature in the room was kept constant at 23°C to maintain an internal bottle temperature of 28/23°C ± 1°C (day/night). Air flow through the bottles was maintained as previously described (Chapter 4).

Each day, for 18 DAS, one seedling was removed from one of the bottles, cleaned of the gelled medium and of the charcoal, rinsed with deionized water and scanned with a scanner for root architectural imaging (Model 3c; Hewlett-Packard). Images of the seedlings were stored as files in a personal computer. Five germinated seedlings (after 24 hr) and five four-day-old seedlings were sampled and prepared for scanning electron microscopy. Specimens to be observed in the scanning electron microscope were fixed in gluteraldehyde, washed in phosphate-buffered saline solution, dehydrated first by gradually increasing alcohol to 100% and then critical-point dried (Postek *et al.*, 1980). The specimens were then mounted with a pair of forceps on a metallic stub and coated with a 2-µm-thick layer of gold. The specimen was observed with a scanning electron microscope (Hitachi-S4000 Field Emission Scanning Electron Microscope).

Results and Discussion

Germinated lettuce seedlings had a visible and distinct hypocotyl and a radicle (Figures 5-1 and 5-2). The hypocotyl region was distinguished from the radicle region by the presence of trichomes, typical appendices of a stem (Figure 5-3). Radicles had root hairs, which can be distinguished from a trichome because they are elongated epidermal cells (Figure 5-4) of the root, whereas trichomes form as separated cells from the epidermal cells of a stem (Hofer, 1991).

Seedlings had not yet unfolded their cotyledons 1 DAS (Figure 5-5, a). Cotyledons were fully expanded and the radicle was differentiated in two zones 2 DAS (Figure 5-5, b). A thicker, short upper radicle was visibly distinguished from a smaller, long, lower radicle. This distinction was more visible 3 (Figure 5-6) and 4 DAS (Figure 5-7).

Basal and lateral roots emerged from the taproot of lettuce seedlings 4 or 5 DAS. In the present research, 'Valmaine' seedlings always had basal roots emerging from the taproot prior to or at the same day of lateral root emergence (Chapters 6 and 7), and after full cotyledon expansion. Basal roots emerged about 1 day prior to lateral roots in bell pepper seedlings growing in a gelled medium, which was after complete cotyledon expansion (Stoffella et

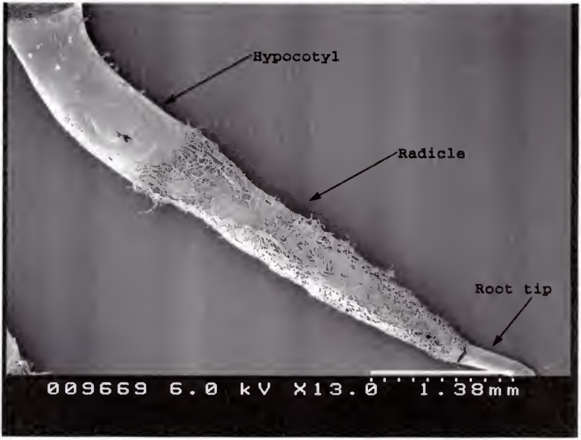

Figure 5-1. Image at the Scanning Electron Microscope showing the different anatomical parts of a germinated lettuce seedling. (Bar = 1.38 mm).

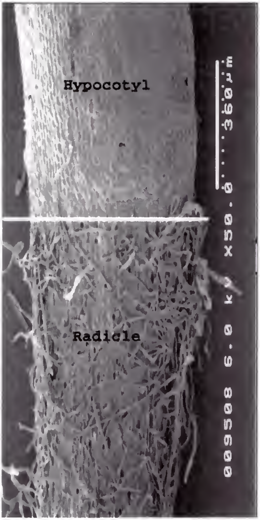

Figure 5-2. Image at the scanning electron microscope showing the lower hypocotyl-upper radicle zone of a germinated lettuce seedling. (Bar = 360 µm).

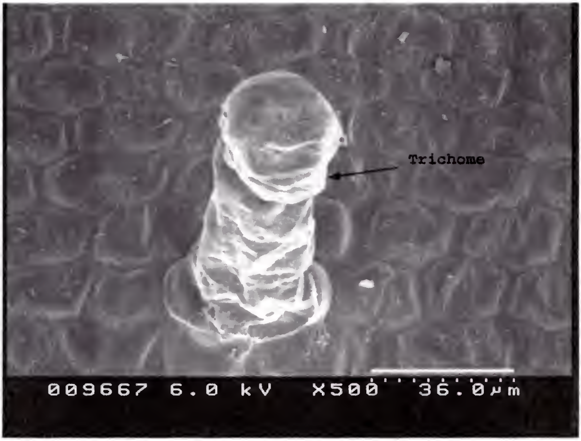

Figure 5-3. Image at the scanning electron microscope showing a trichome in the hypocotyl of a germinated lettuce seedling. (Bar = 36 µm).

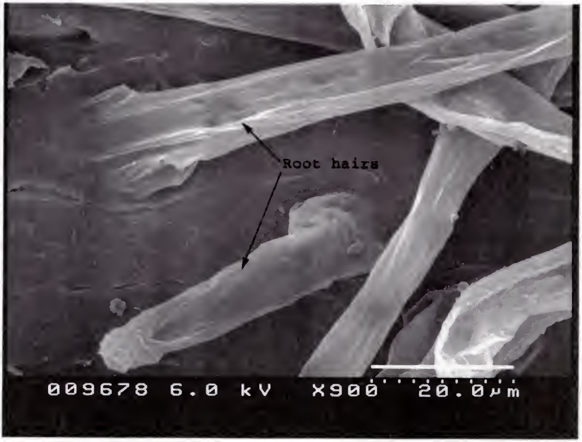

Figure 5-4. Image at the scanning electron microscope showing the presence of root hairs in the radicle of a germinated lettuce seedling. (Bar = 20 µm).

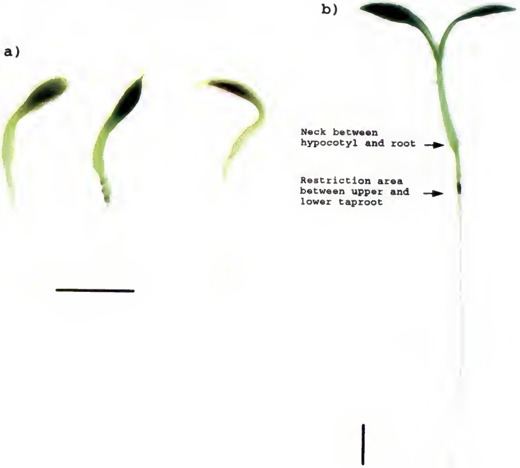
a)
b)
Neck between hypocotyl and root
Restriction area between upper and lower taproot

Figure 5-5. a) Lettuce seedlings 1 DAS with cotyledons not unfolded. Bar = 5 mm. b) Lettuce seedlings 2 DAS with cotyledons unfolded. Bar = 5 mm. A morphological distinction between the upper taproot from which basal roots will arise and the lower taproot from which basal roots will arise is noted. The black stain visible in this region was due to the incomplete removal of the charcoal from the root.

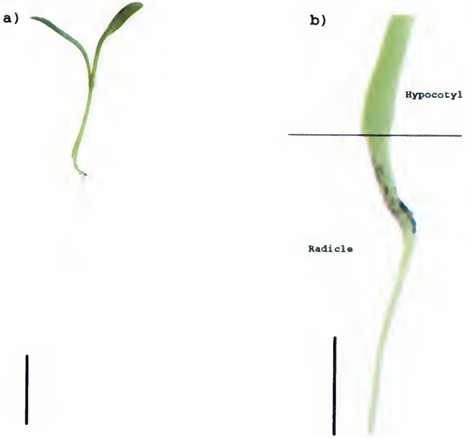

Figure 5-6. Lettuce seedling 3 DAS. a) Whole seedling. Bar = 5 mm. b) Zone of the lower hypocotyl and upper radicle. Bar = 3 mm.

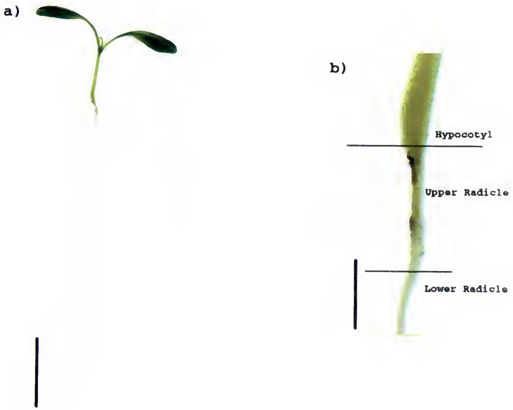

Figure 5-7. Lettuce seedling 4 DAS. a) Whole seedling. Bar = 10 mm. b) Zone of the lower hypocotyl and upper radicle. Bar = 4 mm.

al., 1988). However, lateral roots emerged prior to basal roots in tomato seedlings (Aung, 1982) grown in white quartz sand.

Root analysis with the scanning electron microscope 4 DAS indicated that basal roots originate from the taproot (Figure 5-8). In fact, there was a distinctive presence of root hairs in the area of the taproot where basal roots originated. The upper portion of the taproot (Figures 5-9 and 5-10) was greater in diameter than the lower part of the radicle. In addition, a 'restriction' area separated the two portions of the taproot.

Basal roots were observed to emerge during early seedling growth (4-5 DAS) (Figure 5-11). The zone of the taproot from which basal roots emerged was shorter compared to the rest of the radicle. When lateral roots began to emerge, their number increased rapidly during early seedling growth (Figures 5-12 and 5-13). Lateral root number in lettuce was shown to be greater than basal root number at any given point in time and at the end of the growing period (Chapter 6 and 7).

Weaver and Bruner (1927) reported that primary branches of lettuce grown in soil arose on two opposite sides of the taproot, mostly in the first 300 mm of soil. This pattern was confirmed in the present studies. Basal roots, called by

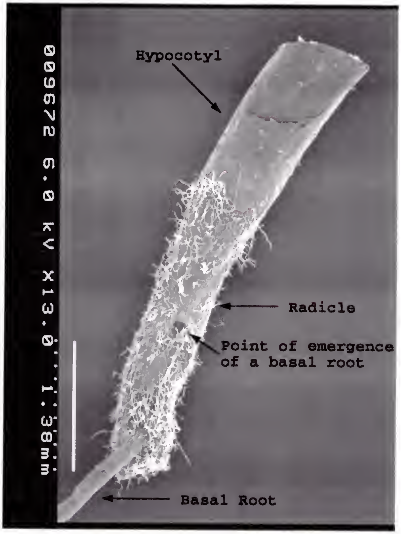

Figure 5-8. Image at the scanning electron microscope showing the lower-hypocotyl and upper-radicle zone of a lettuce seedling 4 DAS. A first basal root had emerged and a second basal root was removed to show its point of emergence. (Bar = 1.38 mm).

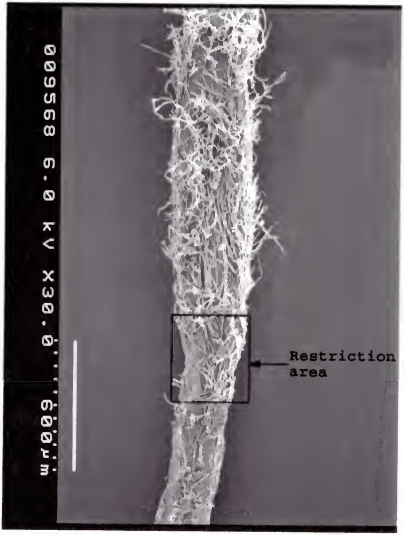

Figure 5-9. Image at the scanning electron microscope showing a portion of the restricted area of the radicle. Lettuce seedling 4 DAS. (Bar = 600 µm).

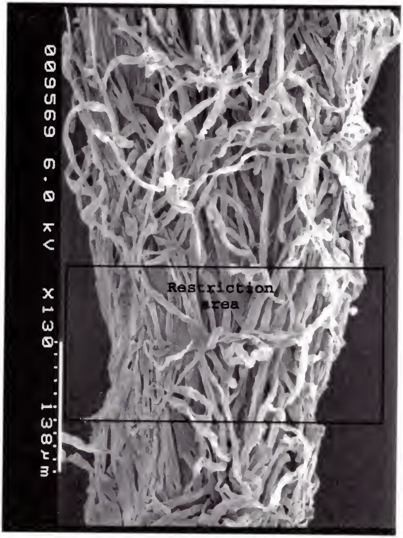

Figure 5-10. Image at the scanning electron microscope showing a portion of the restricted area of the radicle. Lettuce seedling 4 DAS. (Bar = 138 µm).

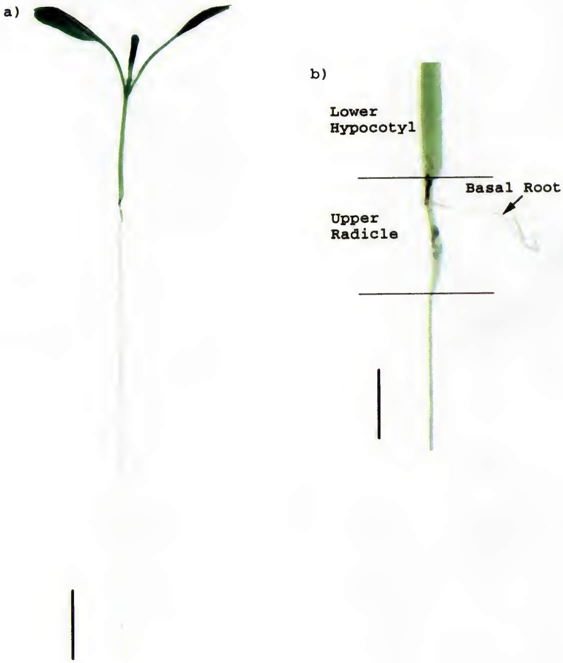

Figure 5-11. Lettuce seedling 5 DAS. a) Whole seedling. Bar = 10 mm. b) Zone of the lower hypocotyl and upper radicle. Bar = 4 mm.

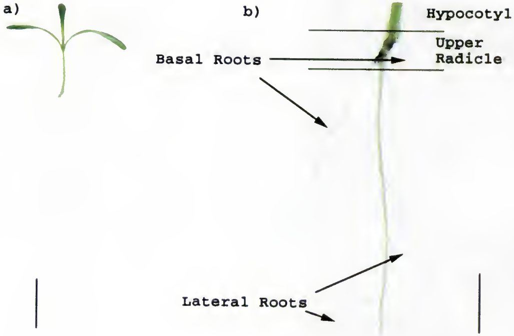

Figure 5-12. Lettuce seedling 6 DAS. a) Whole seedling. Bar = 10 mm. b) Zone of the lower hypocotyl and upper radicle showing basal and lateral root location. Bar = 5 mm.

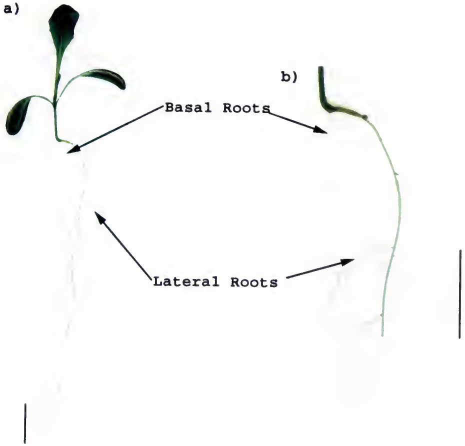

Figure 5-13. Lettuce seedling 7 DAS. a) Whole seedling. Bar = 10 mm. b) Zone of the lower hypocotyl and upper radicle showing basal and lateral root location. Bar = 5 mm.

Weaver and Bruner (1927) and Jackson (1995) as branches arising in the top zone along the taproot, emerged on two sides of the taproot. Lateral roots emerged in a more scattered fashion along the taproot, at about 120° apart (Figure 5-14).

According to Charlton (1996), Weinhold (1967) and Zobel (1986), basal roots are expected to arise acropetally, while lateral roots are expected to arise basipetally from the germinating seedling. Similar patterns were noticed also in lettuce. Basal roots emerged acropetally since older basal roots, emerged close to the restriction area, were longer than the newer and shorter basal roots, and emerging towards the top of the radicle. The opposite pattern occurred in lateral roots. The longer roots were superficial, while new and shorter lateral roots emerged basipetally towards the tip of the radicle (Figures 5-15 and 5-16).

Charlton (1996) reported that additional lateral roots may arise between the emerged lateral roots, particularly in dicots. This phenomenon was observed in the present work (Figures 5-16 and 5-17). Esau (1965) called these lateral roots adventitious. New primary branches originating between older lateral roots were classified as lateral roots. New primary branches emerging between basal roots were observed and were classified as basal roots. The classification of

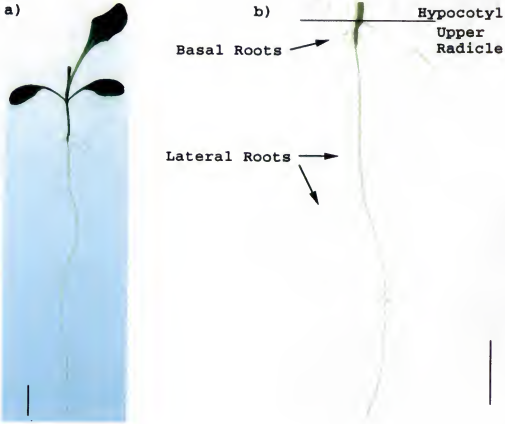

Figure 5-14. Lettuce seedling 8 DAS. a) Whole seedling. Bar = 10 mm. b) Zone of the lower hypocotyl and upper radicle. Note that the basal roots emerged in two opposite sides of the taproot, whereas lateral roots emerged at about 120º apart. Bar = 5 mm.

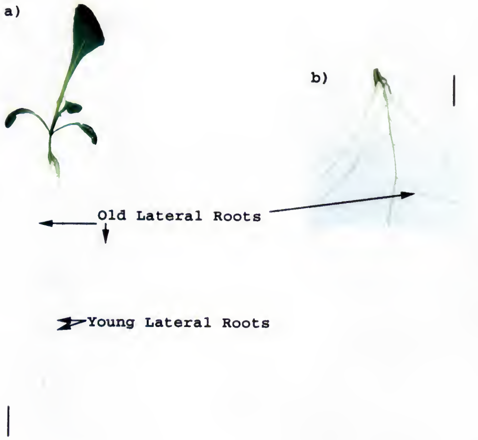

Figure 5-15. Lettuce seedling 9 DAS. a) Whole seedling showing young and old lateral root location. Bar = 10 mm. b) Zone of the and upper radicle showing lateral root location. Bar = 5 mm.

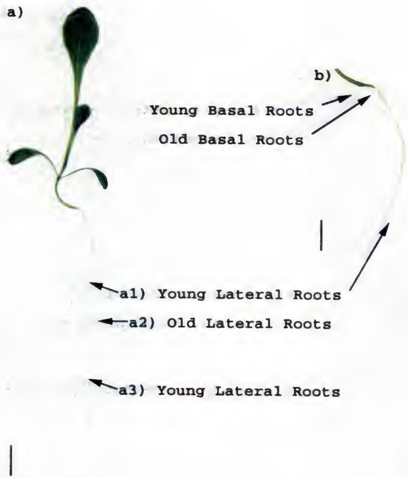

Figure 5-16. Lettuce seedling 10 DAS. a) Whole seedling. Note that between old lateral roots (a2) young lateral roots emerged; other young lateral roots (a3) continued to emerge basipetally. Bar = 10 mm. b) Zone of the upper radicle. Bar = 5 mm.

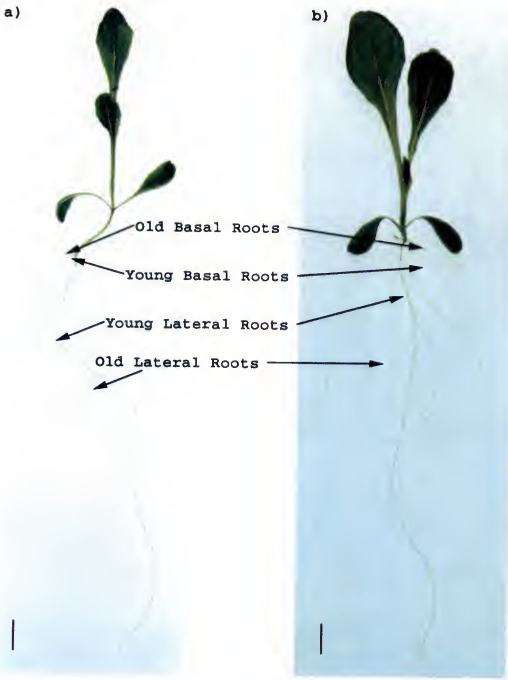

Figure 5-17. a) Lettuce seedling 11 DAS. Bar = 10 mm. b) Lettuce seedling 12 DAS showing younger basal and lateral roots emerging between older basal and lateral roots. Bar = 10 mm.

Zobel (1975) was followed, and the classification as adventitious was given to the branches originated on the stem, above the root zone. However, lettuce seedlings were not observed to form adventitious roots during the 18 days of growth in any of our studies.

Secondary branches began to emerge from the older lateral roots 9 or 10 DAS (Chapters 6 and 7) and closer to the point of initiation of the lateral root that to the lateral root tip (Figures 5-18 and 5-19). A basipetal distribution of new secondary branches along the original lateral root was observed (Figure 5-19). Basal roots formed very few secondary branches in 'Valmaine' lettuce seedlings 18 DAS. Secondary branch differentiation may have an impact on root architecture by increasing the surface area of the total roots available for uptake of water and nutrients, by increasing the plant capacity to explore a greater soil volume, and by giving more support of the shoot to the soil. Genotype can influence the potential of a plant to produce branches, and further research is required to investigate branching differentiation among lettuce genotypes.

Basal and lateral root number and length increased over time (Figures 5-19 and 5-20). Older basal and lateral roots were observed to increase in length as the seedlings grew. Measurements could not be taken, since destructive samplings

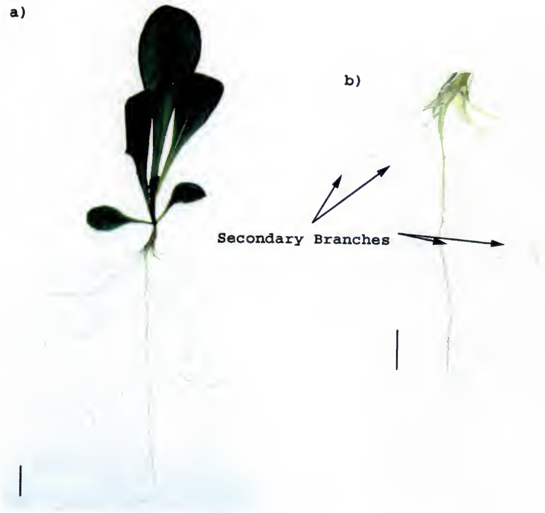

Figure 5-18. Lettuce seedling 13 DAS. a) Whole seedling. Bar = 10 mm. b) Zone of the upper radicle showing secondary branch formation. Bar = 5 mm.

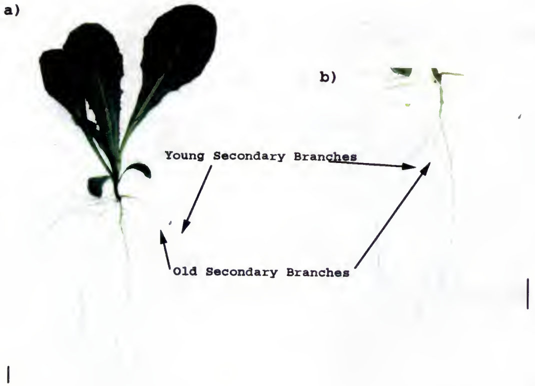

Figure 5-19. Lettuce seedling 14 DAS. a) Whole seedling. Bar = 10 mm. b) Zone of the upper radicle. Pictures show the basipetal formation of the secondary branches. Bar = 5 mm.

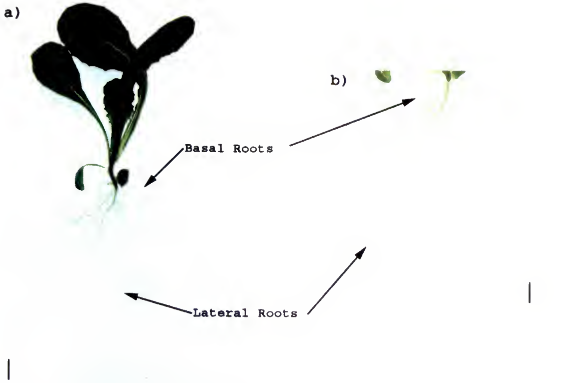

Figure 5-20. Lettuce seedling 15 DAS. a) Whole seedling. Bar = 10 mm. b) Zone of the upper radicle. Pictures show the location of basal and lateral roots. Bar = 5 mm.

could not be done in order to continue to count branch numbers. Lateral roots continued to emerge from the elongating taproot, but not at a constant distance along the taproot (Figures 5-20, 5-21 and 5-22). New roots emerged from the taproot in the zone between older lateral roots and new roots continued to emerge basipetally (Figures 5-20 and 5-23).

Environmental conditions changed root architecture of lettuce seedlings in previous studies (Chapters 6 and 7), but the series of events in root development and architecture could be characterized as a) early radicle elongation from radicle protrusion without any branching until cotyledons were unfolded; b) emergence of basal roots 4 DAS; c) emergence of lateral roots at the same day or later than basal roots, 4 or 5 DAS; d) increased acropetal emergence of basal roots and basipetal emergence of lateral roots in the following days, from 5 to 18 DAS; e) emergence of secondary branches 9 or 10 DAS and arising basipetally from the older lateral roots; f) emergence of basal and lateral roots between older primary roots, contemporary to emergence of basal and lateral roots that followed the original acropetal and basipetal patterns; g) formation of two- to threefold number of lateral roots compared to basal roots. Basal roots emerged at two opposite sides of the

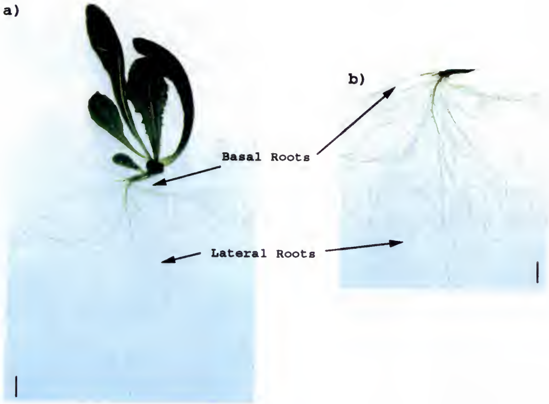

Figure 5-21. Lettuce seedling 16 DAS. a) Whole seedling. Bar = 10 mm. b) Zone of the upper radicle. Pictures show the location of the basal and lateral roots. Bar = 5 mm.

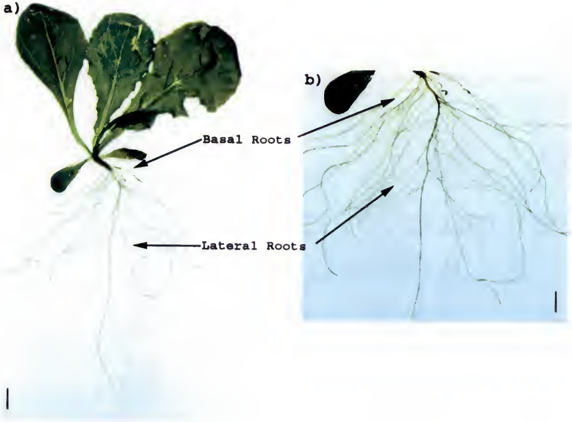

Figure 5-22. Lettuce seedling 17 DAS. a) Whole seedling. Bar = 10 mm. b) Zone of the upper radicle. Pictures show location of basal and lateral roots. Bar = 5 mm.

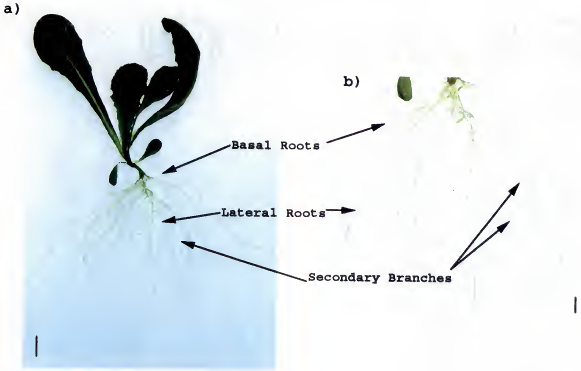

Figure 5-23. Lettuce seedling 18 DAS. a) Whole seedling. Bar = 10 mm. b) Zone of the upper radicle. Pictures show the location of basal and lateral roots and secondary branches. Bar = 5 mm.

short upper taproots, whereas lateral roots emerged at about 120° apart along the taproot, indicating that lateral roots can explore more soil volume for resources than basal roots. Lettuce seedlings did not produce adventitious roots in any of my studies, as was reported in bell pepper by Stoffella et al. (1988).

The present study has indicated that, first, basal and lateral roots formed in lettuce seedlings, as were reported in other dicotyledonous species (Leskovar and Stoffella, 1995; Stoffella *et al.*, 1979; Stoffella et al., 1988; Zobel, 1975. Second, that basal roots in lettuce seedlings originated from the upper taproot. Third, that basal roots emerged prior to or at the same day as lateral roots. Fourth, that basal roots did not produce secondary branches during the first 18 DAS, while lateral roots produced basipetally-emerging secondary branches, starting from the upper and older lateral roots. Finally, that new basal and lateral roots emerged between older primary root branches.

Understanding the architecture of a root system will be useful to determine the plant's potential to explore and to respond to localized availability of the soil resources through its root system. Results of the present study showed that lettuce seedlings developed a distinct root branch formation during the early plant growth, characterized by three types of roots (basal, lateral and secondary lateral roots). An elaborated root branch system has the dual influence on plant growth, both to increase the root surface area available for water and mineral uptake and to give support of the shoot to the soil. This information could be useful when trying to genetically select lettuce cultivars for superior rooting patterns. The demonstration that lateral and basal roots are formed in lettuce may be valuable in modeling rooting patterns of greenhouse- and field-grown plants and, thus, relate these to improved head quality and yield.

Summary

Root architecture can be very important to plant productivity. The spatial distribution in the soil of the root system can determine the capacity of a plant to exploit soil's resources, which are unevenly distributed on the rhizosphere, or subjected to localized depletion by roots. Differences in root branch formation exist in many crops. Lettuce is a horticultural crop which has not been studied for its root architecture. Consequently, the objective of the present study was to describe the root morphology, growth and architecture of young lettuce seedlings grown in a Photosynthetic Whole Plant Culture (PWPC) system.

Lettuce (*Lactuca sativa* L. 'Valmaine') seedlings were grown with gelled medium containing salts of Murashige and Skoog solution, in a Photosynthetic Whole Plant Culture system, under a controlled environment. Air flow was provided to the plants. Seedlings were grown for 18 days after seeding (DAS) under 270 $\mu mol\ m^{-2}s^{-1}$ PPF with a photoperiod of 16 hr and 28/23°C day/night temperature. Germinated and four-day-old seedlings were sampled and scanned with an electron microscope. Every day, young seedlings were sampled and scanned for architectural imaging.

The root architectural analysis has shown that lettuce seedlings produce two types of primary root branches, basal and lateral roots, which originate from two distinct regions of the taproot. Basal roots originated at two opposite sides of a short upper portion of the taproot and did not produce secondary branches during the first 18 DAS. Lateral roots originated at about 120° apart along a longer portion of the taproot and produced secondary branches. Seedlings did not produce adventitious roots.

The series of events in root development in 'Valmaine' lettuce seedlings could be characterized as a) early radicle elongation from radicle protrusion without any branching until cotyledons were unfolded; b) emergence of basal roots

4 DAS; c) emergence of lateral roots at the same day or later than basal roots, 4 or 5 DAS; d) increased acropetal emergence of basal roots and basipetal emergence of lateral roots in the following days, from 5 to 18 DAS; e) emergence of secondary branches 9 or 10 DAS and arising basipetally from the older lateral roots; f) emergence of basal and lateral roots between older primary roots, contemporary to emergence of basal and lateral roots that followed the original acropetal and basipetal patterns; g) formation of two- to threefold number of lateral roots compared to basal roots.

The demonstration that lateral and basal roots are formed in lettuce might be valuable to determine the rooting patterns of field-grown plants and, thus, relate these to improved head quality and yield. This information could be useful when trying to genetically select lettuce cultivars for superior rooting patterns.

CHAPTER 6

SUCROSE AND CO_2 IN A PHOTOSYNTHETIC WHOLE PLANT CULTURE (PWPC) SYSTEM: IS THE SOURCE OF CARBON IMPORTANT TO ENHANCE ROOT SYSTEM GROWTH IN LETTUCE?

Introduction

Sucrose provides carbon skeletons for biosynthetic and respiratory processes during plant growth (Farrar and Williams, 1991). Recently, sucrose has also been hypothesized to have a regulatory role in gene expression, providing a mechanism for adjustment to environmental and/or developmental changes that alter photosynthate supplies (Jan and Sheen, 1996; Koch, 1996; Koch et al., 1996; Kovtun and Daie, 1995). Other morphogenetic processes have been attributed to be controlled by sucrose, such as induction of flowering (Bernier, 1988; Bernier, 1996; Bernier et al., 1993; Corbesier et al., 1996), differentiation of the phloem (Aloni, 1987; Jeff and Northcote, 1967; Wetmore and Rier, 1963; Wright and Northcote, 1972), initiation of root meristems (Street, 1969; van't Hoff et al., 1973) and initiation of roots in callus (Farrar and Williams, 1991; Wright and Northcote, 1972).

Several lines of evidence support the hypothesis that the path of C entry into a plant system or organ can alter its effect at both the substrate and signaling levels. First, the influence of carbon supply on plant growth and respiration can differ with the source and path of delivery for this resource. Correlations have been reported between respiration rate and carbohydrate content of roots, which have been interpreted as indicative of substrate limitation in respiration (Farrar and Williams, 1991). Farrar and Williams (1991) suggested viewing this relationship with caution, hypothesizing that only cytosolic sugars would be expected to correlate with respiration rate, as was observed in their studies with barley roots. Nevertheless, these researchers pointed out that when exogenous sugars are supplied at high concentrations, further stimulation of respiration is minimal. In contrast, growth and respiration increased when photosynthetic input was elevated by raising light intensity or partial pressure of CO_2. Respiratory responses to exogenous sucrose feeding can thus differ from those to CO_2 enrichment, and these differences in turn may be related to altered effects on growth and morphology.

Second, sugar-responsive gene expression can differ depending on whether sucrose is delivered directly to chloroplasts (Sheen, 1990), to leaves (Van Oosten and Besford, 1994), or to seedlings in culture (Brusslan and Tobin, 1992). These studies indicated that sugars can either repress or induce the expression of different genes that encode key enzymes of carbon fixation and metabolism, depending on the experimental system.

Kovtun and Daie (1995) found that sugar beet (*Beta vulgaris* L.) seedlings grown with either 90 or 150 mM sucrose, 90 or 300 mM glucose increased growth rate, photosynthetic rate, and leaf sugar levels, regardless of which sugar was added to the agar-media. The steady-state level of transcripts increased for the small and large subunits of ribulose-1,5 bisphosphate carboxylase/oxygenase (Rubisco) and the cytosolic fructose-1,6 bisphosphatase. The activity of sucrose phosphate synthase increased in the presence of sugars, but the fructose-1,6-bisphosphatase and Rubisco activities remained unchanged. Related evidence suggested that exogenous sugars may have had minimal or no osmotic effects in whole plant experiments since similar growth rates were measured with an osmotically-adjusted medium (300 mM 3-O-methyl glucose) and with sugars. Results from the Kovtun and Daie (1995) studies suggested that, in addition to serving as molecular signals, sugars play important physiological roles in the development of the export capacity to sink tissues. In sugar beet seedlings, the addition of sugar to the culture medium caused accelerated leaf growth, development and sink-to-source transition.

Another line of evidence for differential effects of carbon resources delivered via different routes lies in contrasting growth by autotrophic versus heterotrophic culture systems. Exogenous sucrose is typically indispensable as a carbon and energy source for heterotrophic growth of shoots and plantlets *in vitro* (Kozai et al., 1990). However, seedlings can grow autotrophically if sufficient CO_2 and light are provided. Kozai et al. (1987, 1988a, 1988b) found that explants of carnation, *Cymbidium* and potato cultured *in vitro* under conditions of CO_2 enrichment and high light intensity (150-250 $\mu mol\ m^{-2}s^{-1}$) produced plantlets each having more than twice the mass of those cultured under conditions of low CO_2 and low light intensity (35 $\mu mol\ m^{-2}s^{-1}$). Results were similar regardless of sucrose concentrations in the culture medium. Plant growth was promoted without sucrose or with 1% sucrose in the medium. Net photosynthetic rates per leaf dry weight and per plantlet decreased with increased sucrose concentration in the medium (Kozai, 1989).

Growth and development of *Brassica campestris* L. plantlets cultured in vessels were similar to those of seedlings cultured under the same environmental conditions

(Kozai et al., 1991b). The growth and development of plantlets and seedlings were greater with increased air change and light levels in the vessel, regardless of the sucrose concentration in the culture medium. Low CO_2 concentration in the vessel with a lower number of air changes per hour reduced the net photosynthetic rate of plantlets and seedlings in the vessel, affecting growth and development of both plantlets and seedlings.

Kozai et al. (1988c) reported that growth of tobacco seedlings in culture vessels was improved by CO_2 enrichment, high PPF and sucrose-free medium. Growth was repressed by CO_2-non enrichment, low PPF and sucrose-free medium. However, growth of seedlings was restricted if sucrose was absent from the medium in relatively airtight vessels where photosynthesis would presumably have been limiting (Kozai, 1991a).

Exogenous sucrose application in the growing medium in a Photo-Mixotrophic Whole Plant Culture (PMWPC) system increased plant growth of lettuce seedlings, affected the partitioning of photosynthates between root and shoot, and differentially increased the formation of primary branches in the root system (Chapter 3). A Photosynthetic Whole Plant Culture (PWPC) system has been designed and characterized, and will allow discrepancies in the above studies to be further resolved. The PWPC system allows the plant to utilize CO_2 from the air as a carbon source (Chapter 4). In the following studies, PWPC is used to address the hypotheses that lettuce root system can differentially respond to sucrose feeding and to free air flow, and further, that plant response to sucrose feeding might vary compared to carbon provided by CO_2 in the atmosphere. Results of this work have dual significance, both to applied systems for production and screening of seedlings with extensive root systems, and to fundamental studies of sugar effects on plant growth as influenced by endogenous versus exogenous paths of sucrose delivery.

Materials and Methods

Sucrose Feeding in Plants with Atmospheric Air

Gelled medium 'Phytagel' (Sigma Chemical Co., St. Louis, MO), 2.5 g l^{-1}, was used as a substrate for growing and evaluating root morphology and development of lettuce seedlings from radicle protrusion to 13 days after seeding (DAS). The basal nutrient medium contained inorganic salts according to Murashige and Skoog (1962) (Table 6-1) with or without 30 g l^{-1} (90 mM) of sucrose. The solution pH was adjusted to 6.5 with 1 N NaOH prior to autoclaving. Glass 1-

Table 6-1. Composition of Murashige and Skoog (1962) medium for tissue culture.

Major elements				Minor elements		
Salts	mg/l		mM	Salts	mg/l	µM
NH_4NO_3	1650	N	41.20	H_3BO_3	6.2	100
KNO_3	1900	N,K	18.80	$MnSO_4 \cdot 4H_2O$	22.3	100
$CaCl_2 \cdot 2H_2O$	440	Ca	3.00	$ZnSO_4 \cdot 4H_2O$	8.6	30.0
$MgSO_4 \cdot 7H_2O$	370	Mg,S	1.50	KI	0.83	5.0
KH_2PO_4	170	P,K	1.25	$Na_2MoO_4 \cdot 2H_2O$	0.25	1.0
Na_2EDTA	37.3	Na	0.20	$CuSO_4 \cdot 5H_2O$	0.025	0.1
$FeSO_4 \cdot 7H_2O$	27.8	Fe	0.10	$CoCL_2 \cdot 6H_2O$	0.025	0.1

l bottles (Pyrex, Corning, NY) were prepared by pouring 700 ml of medium and autoclaving them for 35 min at 130° C at 1.1 kg cm^{-2} pressure (15 psi). After autoclaving the medium was cooled to solidify at ambient temperature.

Seeds of lettuce (*Lactuca sativa* L.) cv. Valmaine (Petoseed, Co., CA) were surface-sterilized by soaking them in a solution of 2% NaOCl and 0.1% of Tween 20 for 20 minutes. The seeds were then rinsed five times in sterilized-deionized water, placed in a Petri dish with filter paper no.3 (Whatman International Ltd. Maidstone, England), and moistened with sterilized-deionized water. The seeds were germinated for 24 hr at 20-22°C under continuous fluorescent light (Osram Sylvania Inc., Danvers, MA, 50 $\mu mol \cdot m^{-2} s^{-1}$ PPF). When radicle reached approximately 2 mm, one seedling was transferred in each bottle by placing it at the center top surface of the gelled medium. The seedling was carefully inserted into the medium leaving the upper hypocotyl above the gel surface. Glass bottles were externally covered with a layer of aluminum foil around the medium to block light. Forty bottles were placed in a growth room as previously described (Chapter 4). Temperature in the room was kept constant at 23°C to maintain an internal bottle temperature of 28/23°C ± 1°C (day/night). Air flow through the bottles was accomplished as previously described

(Chapter 4). Plant were grown under 16 hr light and 8 hr dark cycles. The total PPF was 250 µmol $m^{-2}s^{-1}$.

Number of lateral and basal roots and differentiated leaves of each seedling was counted daily for 13 DAS, by visual observation through the bottle. Total root numbers (lateral + basal roots) were counted daily. Secondary branches emerging from the lateral roots were counted starting from the initiation of the first branch root (approximately 10 DAS). At plant harvest, root and shoot dry weights were obtained after oven drying of each seedling for 96 hr at 60°C. Taproot length was measured for each plant with a ruler with an approximation of 1 mm.

The experimental design consisted of a randomized complete block design with 4 blocks of 5 observations each. Treatments consisted of 2 levels of sucrose (0 and 30 g l^{-1}). Data were analyzed using a SAS statistical system (SAS Institute Inc., 1989). All data were taken daily and submitted to the analysis of variance of repeated measurements to investigate plant growth response over time (Littell, 1989; Chapter 8). All χ^2 tests of Mauchly's criterion were highly significant and the G-G and H-F epsilons were lower than 1 (Appendix A8-17), consequently the G-G adjusted P values were used (Greenhouse and Geisser, 1959) for all interactions with time. Data over time for growth trend analyses were plotted for each sucrose level. Trend analyses were performed by considering two main growth phases: 1) Phase of initial growth, from the protrusion of lateral and/or basal roots (4 DAS to 7 DAS); 2) Phase of continuation of growth (7 to 13 DAS). In the case of lateral secondary branches only one Phase was considered, from the initiation of secondary root branches (10 DAS) to harvest (13 DAS). Linear slopes of the line segments for the 2 phases were computed and analyzed. Data taken at the termination of the experiment were submitted to the analysis of variance. Least Squares Means (LSM) and Standard Errors (SE) were calculated for each measured variable.

Sucrose Feeding in Plants with Atmospheric or CO_2-enriched Air

Bottles were filled with 650 ml of medium as previously described. Another 50-ml layer of medium which contained 3 g l^{-1} activated charcoal (Grand Island Biological Company, Grand Island, NY) was poured over the surface of the gel to reduce light in the root zone and avoid Fe-catalyzed photooxidation of EDTA present in the substrate (Hangarter and Stasinopoulos, 1991). The external surfaces of the bottles were covered with aluminum foil as previously discussed. Plants were grown for 18 DAS.

The experimental design consisted of a randomized complete block design with 2 blocks of 5 observations each. Treatments were 2 levels of sucrose (0 and 30 g l^{-1}) and 2 concentrations of CO_2 in the air (350 µmol mol^{-1} or standard atmospheric air, and 2000 µmol mol^{-1} or CO_2-enriched air) in a factorial combination. Data were analyzed as previously described. Also in this study all χ^2 tests of Mauchly's criterion were highly significant and the G-G and H-F epsilons were lower than 1 (Appendix A8-18), consequently the G-G adjusted P values were used (Greenhouse and Geisser, 1959) for all interactions with time. Trend analyses were performed by considering two main growth phases: 1) Phase of initial growth (4 DAS to 7 DAS); 2) Phase of continuation of growth (7 to 18 DAS). In the case of lateral secondary branches only one Phase was considered, from the initiation of branches (10 DAS) to harvest (18 DAS). Estimated linear slopes of the line segments for the two phases were computed and analyzed. At the completion of the experiment data were submitted to the analysis of variance. Least Squares Means (LSM) and Standard Errors (SE) were calculated for each measured variable.

Results and Discussion

Sucrose Feeding in Plants with Atmospheric Air

Plant root growth and development

The sucrose main effect was analyzed with the univariate F test and was not significant in any variable (Tables 6-2 and 6-3). The sucrose by time interaction was significant on lateral, total and secondary root numbers, but not on basal root and leaf numbers. Lateral and basal roots emerged from the seedling taproot 4 DAS. Cotyledons of all seedlings were already fully expanded. At that time, means of lateral roots were 0.3 and 1.0 in plants grown with or without sucrose, respectively (Figure 6-1). Basal roots were 0.6 and 0.8 (Figure 6-2).

Sucrose affected the number of lateral roots in interaction with time (Table 6-2, Figure 6-1). During the first phase of growth, from 4 to 7 DAS, sucrose-fed seedlings had a greater linear increase of lateral root number than nonsucrose-fed seedlings (Tables 6-4 and 6-5). During the second phase of growth, from 7 to 13 DAS, linear trends of lateral root formation between sucrose-fed and nonsucrose-fed seedlings did not differ, resulting, 13 DAS, in seedlings with a similar number of lateral roots, regardless of sucrose concentration in the medium.

Table 6-2. Univariate analysis of variance and G-G adjustments of repeated measures for lateral, basal and total root number of lettuce seedlings grown 13 DAS in medium containing either 0 or 30 g l^{-1} sucrose.

		Root number					
		Lateral		Basal		Total	
Source of variation	DF	Univariate F Test	G - G Adjust.	Univariate F Test	G - G Adjust.	Univariate F Test	G - G Adjust.
Block	3						
Sucrose	1	0.2418		0.0718		0.1585	
Error	3						
Sampling error	26						
Analysis over time							
Time	9	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Time * Block	27	0.0460	0.1774	0.0209	0.1260	0.0129	0.1137
Time * Sucrose	9	0.0001	0.0015	0.0148	0.0948	0.0001	0.0005
Error	234						

Table 6-3. Univariate analysis of variance and G-G adjustments of repeated measures for number of leaves and lateral secondary branches of lettuce seedlings grown 13 DAS in medium containing either 0 or 30 g l^{-1} sucrose.

		Number of				
		Leaves			Secondary Branches	
Source of variation	DF	Univariate F Test	G - G Adjustment		Univariate F Test	G - G Adjustment
Block	3					
Sucrose	1	0.4150			0.2188	
Error	3					
Sampling error	26					
Analysis over time				DF		
Time	9	0.0001	0.0001	3	0.0001	0.0001
Time * Block	27	0.0001	0.0001	9	0.0001	0.0035
Time * Sucrose	9	0.0371	0.0797	3	0.0014	0.0105
Error	234			78		

Table 6-4. P values of the different segments (slopes) of trend analysis of lateral, basal and total root number, number of leaves and lateral secondary branches of lettuce seedlings grown 13 DAS in medium containing either 0 or 30 g l^{-1} sucrose. (Phase 1 = 4 to 7 DAS; Phase 2 = 7 to 13 DAS; Phase = 10 to 13 DAS).

	Lateral Roots		Basal Roots	
Treatments	Phase 1	Phase 2	Phase 1	Phase 2
Sucrose	0.0465	0.5024	0.0491	0.6379

	Total Roots		Number of Leaves	
	Phase 1	Phase 2	Phase 1	Phase 2
Sucrose	0.0193	0.5995	0.1404	0.1974

	Secondary Branches
	Phase
Sucrose	0.1718

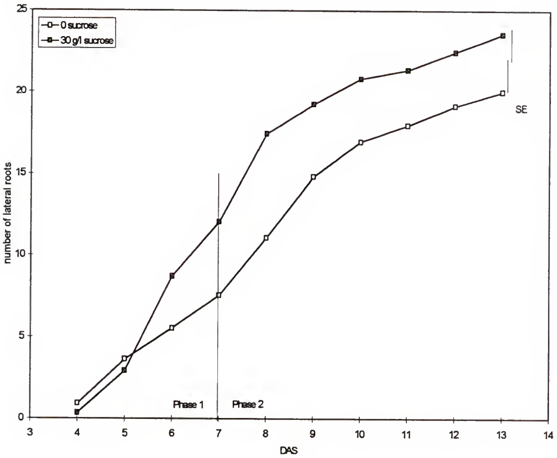

Figure 6-1. Number of lateral roots from 4 to 13 DAS in lettuce seedlings grown in medium containing either 0 or 30 g l^{-1} sucrose.

Table 6-5. Least Squares Means (LSM) and relative Standard Errors (SE) of the slopes for the response of lettuce seedlings to 2 sucrose levels in the medium during the two phases of growth. Effects on lateral root number. (Phase 1 = 4 to 7 DAS; Phase 2 = 7 to 13 DAS).

	Sucrose		
	0	30 g l^{-1}	
Growth phase	LSM	LSM	SE
Phase 1	1.1	2.0	0.2
Phase 2	2.0	1.7	0.3

Consequently, increased lateral root number was affected by sucrose only during the early days of lettuce seedling growth, after which seedlings could develop their photosynthetic capacity by using the CO_2 provided in the air.

Estimated slopes of the linear trends for basal root numbers differed between sucrose-fed and nonsucrose-fed seedlings during Phase 1 (Tables 6-4 and 6-6). More basal roots were produced in the seedlings when exogenous sucrose was added to the medium. During Phase 2 of growth slopes of the trends between sucrose-fed and nonsucrose-fed seedlings did not differ (Table 6-4). As in the case of lateral roots, after the early stages of growth (from 4 to 7 DAS), lettuce basal root formation did not respond to added sucrose. At 13 DAS sucrose-fed and nonsucrose-fed seedlings did not differ in terms of basal root number (Figure 6-2). This would confirm the hypothesis that when sufficient CO_2 is provided to the plants, sucrose in the growing medium is no longer required for root growth. The air flow into the bottle may have allowed the plants sufficient carbon supply from CO_2 in the air, allowing photosynthetic activity, and consequently, reducing the exogenous sucrose effect on growth. Stoffella et al. (1988) found that in pepper seedlings grown for 10 days in a PMWPC system, exogenous sucrose was used by the

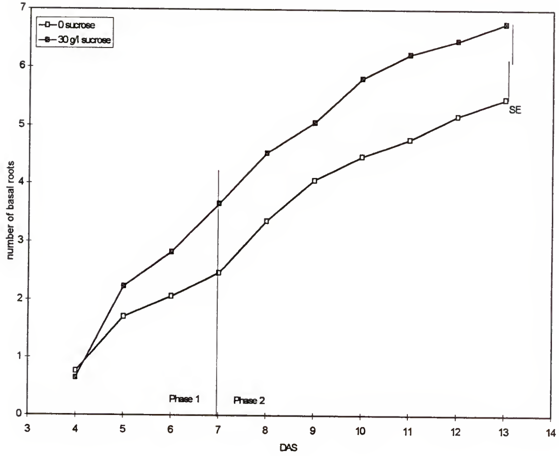

Figure 6-2. Number of basal roots from 4 to 13 DAS in lettuce seedlings grown in medium containing either 0 or 30 g l^{-1} sucrose.

Table 6-6. Least Squares Means (LSM) and relative Standard Errors (SE) of the slopes for the response of lettuce seedlings to 2 sucrose levels in the medium during the two phases of growth. Effects on basal root number. (Phase 1 = 4 to 7 DAS; Phase 2 = 7 to 13 DAS).

	Sucrose		
	0	30 g l^{-1}	
Growth phase	LSM	LSM	SE
Phase 1	0.3	0.5	0.1
Phase 2	0.5	0.5	0.1

plant preferentially for lateral root formation rather than for basal root or taproot growth. Enhanced lateral and basal root growth and taproot length with exogenous sucrose application have been shown in lettuce seedlings grown in a PMWPC for 9 DAS (Chapter 3). Path of carbohydrate delivery was in both cases through the root system. The present study indicated that after an initial burst in root growth, autotrophic seedlings use their preferential path carbon substrate, i.e. CO_2.

Sucrose in the medium significantly affected total lettuce seedling root number, in interaction with time (Table 6-2, Figure 6-3). Sucrose in the medium induced a greater linear increase of total root number in lettuce seedlings during Phase 1 (Tables 6-4 and 6-7) than sucrose-free medium, as it happened for lateral and basal root formation. Similarly to lateral and basal root formation, total root number was not affected by sucrose during Phase 2. At 13 DAS sucrose-fed seedlings had a greater number of total roots than nonsucrose-fed seedlings (Figure 6-3).

Secondary branches measured from the lateral roots emerged 10 DAS. An interaction time x sucrose for secondary branch formation occurred, indicating that with time secondary branches were suppressed when plants were grown with sucrose, but 13 DAS the number of secondary branches

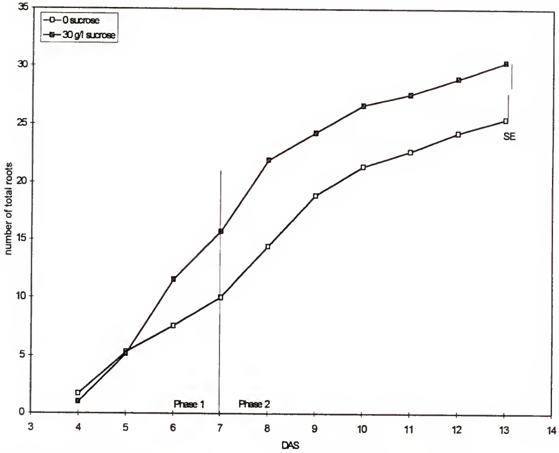

Figure 6-3. Number of total roots from 4 to 13 DAS in lettuce seedlings grown in medium containing either 0 or 30 g l^{-1} sucrose.

Table 6-7. Least Squares Means (LSM) and relative Standard Errors (SE) of the slopes for the response of lettuce seedlings to 2 sucrose levels in the medium during the two phases of growth. Effects on total root number. (Phase 1 = 4 to 7 DAS; Phase 2 = 7 to 13 DAS).

	Sucrose		
	0	30 g l^{-1}	
Growth phase	LSM	LSM	SE
Phase 1	1.4	2.5	0.2
Phase 2	2.5	2.2	0.4

did not differ between sucrose-fed and nonsucrose-fed seedlings (Table 6-3) (Figure 6-4). Thus, sucrose enhanced initiation of both lateral and basal roots, but did so to different degrees, and mainly during the very early days of growth. The enhanced growth occurred in sucrose-fed plants was then maintained constant throughout the following days.

The first true leaf differentiated 4 DAS (Figure 6-5). Leaf number was not affected by an interaction time x sucrose (Table 6-3). During the 2 phases of growth, estimated slopes of the leaf formation linear trends did not differ between sucrose-fed and nonsucrose-fed seedlings (Table 6-4). This would suggest that leaf differentiation was not attributable to an exogenous application of sucrose.

Plant biomass 13 days after seeding

Sucrose did not enhance root and total dry mass, 13 DAS (Table 6-5), but enhanced shoot dry mass. Shoot dry mass was 25 mg in sucrose-fed seedlings, while it was 19 mg in nonsucrose-fed seedlings. The greater shoot dry mass of sucrose-fed seedlings, however, did not result in a decreased root to shoot ratio in sucrose-fed plants. Sucrose did not affect taproot length. An increase in shoot dry mass 9 DAS in sucrose-fed 'Valmaine' seedlings was reported in a previous study using a PMWPC system (Chapter 3). In that study root dry mass increased in sucrose-fed seedlings,

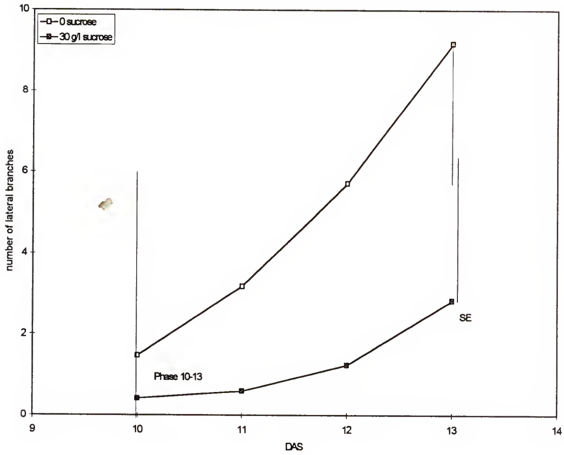

Figure 6-4. Number of lateral secondary branches from 10 to 13 DAS in lettuce seedlings grown in medium containing either 0 or 30 g l^{-1} sucrose.

Table 6-8. Least Squares Means (LSM) and relative Standard Errors (SE) of the slopes for the response of lettuce seedlings to 2 sucrose levels in the medium during the two phases of growth. Effects on lateral secondary branches. (Phase = 10 to 13 DAS).

	Sucrose		
	0	30 g l^{-1}	
Growth phase	LSM	LSM	SE
Phase	1.3	0.4	0.4

leading to a greater root to shoot ratio. In the present study, exogenous sucrose application did not result in a carbon accumulation in the roots, whereas it resulted in an increased carbon accumulation in the shoot. Shoot growth was enhanced in sweet potato plantlets (Hattori et al., 1990) and in sugar beet seedlings with sucrose in the medium (Kovtun and Daie, 1995). Root dry mass was also increased by sucrose feeding in sugar beet seedlings (Kovtun and Daie, 1995).

The present study indicated that 'Valmaine' lettuce seedling growth responded to exogenous sucrose application only in the very early days of growth. Sucrose was loaded from the medium by the roots, thus enhancing primary branch formation. After about 7 days of growth, seedlings developed their photosynthetic capacity by utilizing the CO_2 in the air as a carbon source, and sucrose was no longer affecting primary branch formation. Kovtun and Daie (1995) found that 7 day old leaves of sucrose-fed sugar beet seedlings showed an advanced stage of development compared to the nonsucrose-fed seedlings, including a decline in leaf sink demand and a transition to full-source status. Sugar-induced differences were not noted in 13 day old sucrose-fed or nonsucrose-fed seedlings. The authors suggested that leaves of seedlings grown in sugar-free medium can attain full-source status at

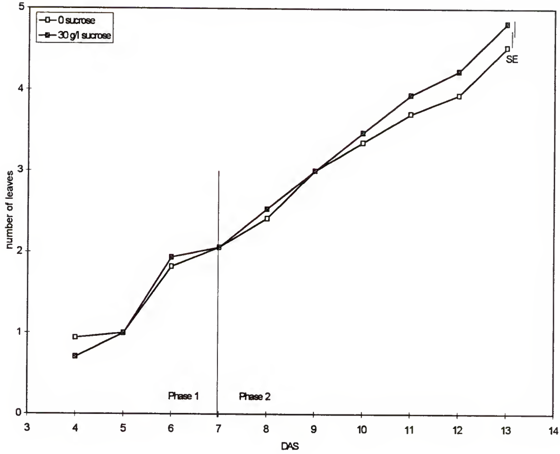

Figure 6-5. Number of differentiated leaves from 4 to 13 DAS in lettuce seedlings grown in medium containing either 0 or 30 g l^{-1} sucrose.

Table 6-9. Least Squares Means (LSM) and relative Standard Errors (SE) of the slopes for the response of lettuce seedlings to 2 sucrose levels in the medium during the two phases of growth. Effects on leaf number. (Phase 1 = 4 to 7 DAS; Phase 2 = 7 to 13 DAS).

	Sucrose		
	0	30 g l^{-1}	
Growth phase	LSM	LSM	SE
Phase 1	0.2	0.2	0.01
Phase 2	0.4	0.4	0.04

Table 6-10. Means with significant probability for differences in the response of lettuce seedlings to sucrose feeding in the medium for dry mass, taproot length and root:shoot ratio 13 DAS.

	Dry Mass (mg)			Taproot Length (mm)	Root:Shoot Ratio
Sucrose	Root	Shoot	Total		
0	3.1	19	22	88	0.16
30 g l^{-1}	3.5	25	28	81	0.14
Probability	0.4957	0.0428	0.0640	0.2483	0.0522

a later stage. Consequently, sucrose supplied to whole plants via the root system may become the not preferential carbon substrate for the plant growing system when sink-to-source transition has occurred in the leaves.

Sucrose Feeding in Plants with Atmospheric or CO_2-enriched Air

Plant root growth and development

Overall mean of lateral root number was increased by 2000 µmol mol^{-1} CO_2 compared to 350 µmol mol^{-1} (12.28 *vs.* 10.02, respectively, Table 6-11) and with sucrose-free medium compared to sucrose in the medium (14.98 *vs.* 8.9, Table 6-11 and Appendix B-1). The overall mean of lateral secondary branches was increased by 2000 µmol mol^{-1} CO_2, more evidently when sucrose was not present in the medium (interaction significant, Table 6-12). The greatest overall mean of lateral root number was reached with 2000 µmol mol^{-1} CO_2 and sucrose-free medium (7.34). According to G-G Adjusted P values, the interaction between time, sucrose and CO_2 did not occur in any variable (Tables 6-11 and 6-12). Lateral and total root numbers were affected by an interaction sucrose x time. Numbers of basal roots, lateral secondary branches and leaves were not affected. Numbers of basal and total roots, of secondary branches and of leaves

Table 6-11. Univariate analysis of variance and G-G adjustments of repeated measures for lateral, basal and total root number of lettuce seedlings grown 18 DAS.

		Root number					
		Lateral		Basal		Total	
Source of variation	DF	Univariate F Test	G - G Adjust.	Univariate F Test	G - G Adjust.	Univariate F Test	G - G Adjust.
Block	1						
CO_2	1	0.0084		0.2163		0.0873	
Sucrose	1	0.0074		0.9350		0.3222	
Sucrose * CO_2	1	0.1652		0.8123		0.8998	
Error	3						
Sampling error	32						
Analysis over time							
Time	14	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Time * Block	14	0.0001	0.0153	0.0001	0.0272	0.0001	0.0057
Time * CO_2	14	0.0044	0.0897	0.0001	0.0173	0.0001	0.0067
Time * Sucrose	14	0.0001	0.0001	0.1980	0.2753	0.0001	0.0001
Time * CO_2 * Sucrose	14	0.5976	0.4496	0.9989	0.7718	0.9465	0.6319
Error	490						

Table 6-12. Univariate analysis of variance and G-G adjustments of repeated measures for number of leaves and lateral branches of lettuce seedlings grown 18 DAS.

		Number of				
		Leaves			Secondary Branches	
		Univariate	G - G		Univariate	G - G
Source of variation	DF	F Test	Adjustment		F Test	Adjustment
Block	1					
CO_2	1	0.2509			0.0043	
Sucrose	1	0.9101			0.0226	
Sucrose * CO_2	1	0.9614			0.0261	
Error	3					
Sampling error	32					
Analysis over time				DF		
Time	14	0.0001	0.0001	8	0.0001	0.0001
Time * Block	14	0.0001	0.0001	8	0.7483	0.5183
Time * CO_2	14	0.0001	0.0021	8	0.0001	0.0228
Time * Sucrose	14	0.6244	0.5537	8	0.0457	0.1469
Time * CO_2 * Sucrose	14	0.3900	0.3885	8	0.1630	0.2352
Error	490			280		

were affected by an interaction time x CO_2. Lateral root numbers were not.

Lateral roots did not emerge from the taproot before 5 DAS (Figure 6-6). Estimated slopes of lateral root formation trends differed between seedlings provided of 2000 µmol mol^{-1} CO_2 and seedlings provided of 350 µmol mol^{-1} CO_2 from 4 to 7 DAS (Phase 1) (Table 6-13). More lateral roots emerged from the seedlings when CO_2 enrichment occurred. The greatest rate of lateral root formation, 1.6 roots per day, was reached by seedlings grown with sucrose and 2000 µmol mol^{-1} CO_2 (Table 6-14). Slopes of lateral root formation trends differed between sucrose-fed and nonsucrose-fed seedlings. Sucrose in the medium enhanced lateral root formation, compared to sucrose-free medium. Estimated slopes of lateral root formation trends differed between sucrose-fed and sucrose-fed seedlings from 7 to 18 DAS (Phase 2). During this phase nonsucrose-fed seedlings produced more lateral roots than sucrose-fed seedlings, regardless of the CO_2 amount in the air (Table 6-13). The greatest rate of lateral root formation, 0.9 roots per day, was reached by seedlings grown without sucrose and 2000 µmol mol^{-1} CO_2. Enrichment of CO_2 did not affect lateral root formation in Phase 2. At 18 DAS, 20.5 lateral roots were formed by

Table 6-13. P values of the different segments (slopes) of trend analysis of lateral, basal and total number of roots, number of leaves and lateral branches of lettuce seedlings DAS 4 through 18. (Phase 1 = 4 to 7 DAS; Phase 2= 7 to 18 DAS; Phase = 10 to 18 DAS).

	Lateral Roots		Basal Roots	
Treatments	Phase 1	Phase 2	Phase 1	Phase 2
Co_2	0.0124	0.5814	0.1934	0.3607
Sucrose	0.0056	0.0048	0.4606	0.4683
Co_2 * Sucrose	0.1016	0.6564	0.8560	0.7980
	Total Roots		Number of Leaves	
	Phase 1	Phase 2	Phase 1	Phase 2
Co_2	0.0283	0.1606	0.6971	0.2278
Sucrose	0.3791	0.0230	0.1215	0.9381
Co_2 * Sucrose	0.2647	0.5242	0.2888	0.6523

	Secondary Branches
	Phase
Co_2	0.2973
Sucrose	0.3929
Co_2 * Sucrose	0.5029

nonsucrose-fed seedlings with 350 µmol mol^{-1} CO_2, 23.3 lateral roots were formed by nonsucrose-fed seedlings with 2000 µmol mol^{-1} CO_2, whereas 12.4 were formed by sucrose-fed seedlings with 350 µmol mol^{-1} CO_2 and 17.0 were produced by sucrose-fed seedlings grown with 2000 µmol mol^{-1} CO_2 (Figure 6-6).

Thus, the plant response to sucrose feeding for lateral root formation was similar between the previous (Tables 6-2 and 6-4) and the present study with regard to Phase 1 of growth. During Phase 2, lateral root formation was repressed by sucrose feeding.

Cotyledons of all seedlings were already fully expanded when the first basal roots emerged from the radicle (4 DAS). Seedlings grown with 2000 µmol mol^{-1} CO_2 produced more basal roots than seedlings grown with normal air from 4 to 18 DAS (Table 6-11, Figure 6-7). This was not the case for lateral root number. Basal root numbers were constantly similar between treatments during Phase 1 and 2 (Tables 6-13 and 6-15). Eighteen DAS less basal roots were formed in nonsucrose-fed seedlings and grown with 350 µmol mol^{-1} CO_2 than in seedlings grown with 2000 µmol mol^{-1} CO_2, regardless of the sucrose addition in the medium. Sucrose did not affect basal root number from 4 to 18 DAS (Table 6-11).

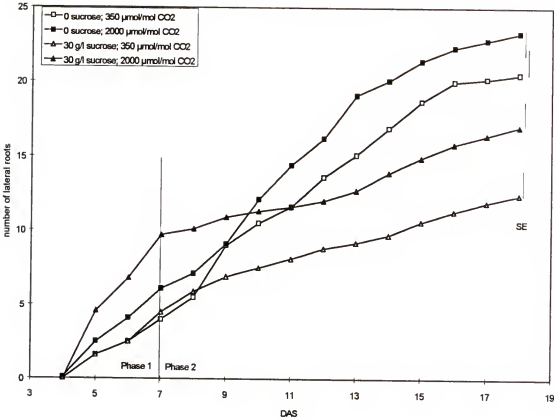

Figure 6-6. Number of lateral roots from 4 to 18 DAS in lettuce seedlings grown with or without sucrose in the medium, with normal air (350 µmol mol^{-1} CO$_2$) or CO$_2$-enriched air (2000 µmol mol^{-1} CO$_2$).

Table 6-14. Least Squares Means (LSM) and relative Standard Errors (SE) of the slopes for the response of lettuce seedlings to 2 sucrose levels in the medium and 2 CO$_2$ concentrations in the air during the two phases of growth. Effects on lateral root number. (Phase 1 = 4 to 7 DAS; Phase 2= 7 to 18 DAS).

	Sucrose (g l^{-1})				
	0		30		
	CO$_2$ (µmol mol^{-1})		CO$_2$ (µmol mol^{-1})		
	350	2000	350	2000	
Growth phase	LSM	LSM	LSM	LSM	SE
Phase 1	0.6	1.0	0.7	1.6	0.3
Phase 2	0.8	0.9	0.3	0.3	0.1

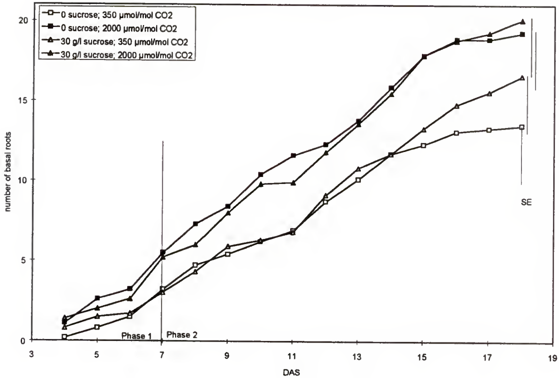

Figure 6-7. Number of basal roots from 4 to 18 DAS in lettuce seedlings grown with or without sucrose in the medium, with normal air (350 µmol mol^{-1} CO_2) or CO_2-enriched air (2000 µmol mol^{-1} CO_2).

Table 6-15. Least Squares Means (LSM) and relative Standard Errors (SE) of the slopes for the response of lettuce seedlings to 2 sucrose levels in the medium and 2 CO_2 concentrations in the air during the two phases of growth. Effects on basal root number. (Phase 1 = 4 to 7 DAS; Phase 2 = 7 to 18 DAS).

	Sucrose (g l^{-1})				
	0		30		
	CO_2 (µmol mol^{-1})		CO_2 (µmol mol^{-1})		
	350	2000	350	2000	
Growth phase	LSM	LSM	LSM	LSM	SE
Phase 1	0.5	0.7	0.3	0.6	0.2
Phase 2	0.5	0.7	0.6	0.7	0.1

Thus, carbon supply to the seedlings affected differently lateral and basal root formation. In fact, exogenous sucrose increased lateral root formation from 4 to 7 DAS and after that suppressed lateral root formation. Exogenous sucrose did not increase basal root formation. Conversely, supply of CO_2 increased lateral root formation during Phase 1 of plant growth, and had a constant effect on increasing basal root formation throughout the entire growth period. This finding is an indication that between exogenous and endogenous paths of sucrose delivery a differential growth effect occurred.

The plant total root number (Figure 6-8) was significantly affected by a time x CO_2 interaction and by a time x sucrose interaction (Table 6-11). An enhancement in total root formation with 2000 µmol mol^{-1} CO_2 was particularly evident between 4 and 7 DAS (Phase 1, P=0.0283), and it was mainly due to an increase in lateral root number (Tables 6-13 and 6-14). Linear trends of total root formation did not differ between seedlings grown with 350 µmol mol^{-1} CO_2 and 2000 µmol mol^{-1} CO_2, form 7 to 18 DAS (Phase 2) (Table 6-13). Analysis of the time x sucrose interaction for total root numbers indicated that sucrose did not affect total root formation during Phase 1 of plant growth, but sucrose suppressed total root formation during

Phase 2 of plant growth. The least increase in total root numbers was reached by sucrose-fed seedlings grown with 350 µmol mol^{-1} CO_2 (Table 6-16), resulting 18 DAS in seedlings with less total root number than those grown with 2000 µmol mol^{-1} CO_2, regardless of the sucrose presence in the medium (Figure 6-8).

During the first study, after an early enhancement in total root formation due to exogenous sucrose, the sucrose effect was nullified. During this second study sucrose suppressed root formation. The apparent discrepant result between the two studies could be explained in two ways. First, in this second study plants were grown for 18 DAS, 5 days longer than in the first study. Consequently, sucrose response was analyzed for a longer period of time. In fact, in the second study total root number 13 DAS was similar to the total root number in the previous study. Second, in the second study a CO_2 treatment was added. When grown for a longer period of time, lettuce seedlings apparently preferentially used CO_2 in the air as a carbon source compared to adding sucrose as a carbon source in the medium. Interestingly, a long-term effect of sucrose caused a suppression of branch formation. Further investigations are needed to fully understand the long-term sucrose effect on root growth of autotrophic seedlings.

An interaction between time and CO_2 enrichment occurred on the number of lateral secondary branches (Table 6-12) (Figure 6-9). With 2000 µmol mol^{-1} CO_2, lateral secondary branch formation increased from 10 to 18 DAS. Sucrose by time interaction did not affect branch number. Eighteen DAS more branches emerged in nonsucrose-fed seedlings supplied with 2000 µmol mol^{-1} CO_2 (17.0) than in the other seedlings (Figure 6-9). Branch numbers were less in sucrose-fed (7.4/9.9 350/2000 µmol mol^{-1} CO_2, respectively) or nonsucrose-fed seedlings grown with 350 µmol mol^{-1} CO_2 (7.5).

Carbon paths into the lettuce seedlings affected differently secondary branch formation. In fact, exogenous sucrose application suppressed secondary branch formation from 10 to 13 DAS in the first study, when 350 µmol mol^{-1} CO_2 were supplied (standard air) (Table 6-3). In the second study, sucrose-free medium and CO_2 enrichment led to a greater number of secondary branches 18 DAS.

Leaf number was affected by an interaction time x CO_2 (Table 6-12). The interaction was due to the time effect, that is leaf timing of formation between seedlings grown with 350 and 2000 µmol mol^{-1} CO_2 differed according to the stage of seedling development. In fact, no differences in leaf number were detected between seedlings grown with 2000 and 350 µmol mol^{-1} CO_2, during the first 7 days. At 8, 9 and

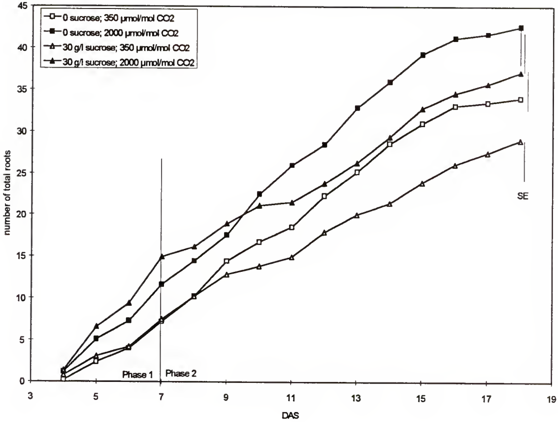

Figure 6-8. Number of total roots from 4 to 18 DAS in lettuce seedlings grown with or without sucrose in the medium, with normal air (350 µmol mol^{-1} CO_2) or CO_2-enriched air (2000 µmol mol^{-1} CO_2).

Table 6-16. Least Squares Means (LSM) and relative Standard Errors (SE) of the slopes for the response of lettuce seedlings to 2 sucrose levels in the medium and 2 CO_2 concentrations in the air during the two phases of growth. Effects on total root number. (Phase 1 = 4 to 7 DAS; Phase 2 = 7 to 18 DAS).

	Sucrose ($g\ l^{-1}$)				
	0		30		
	CO_2 (µmol mol^{-1})		CO_2 (µmol mol^{-1})		
	350	2000	350	2000	
Growth phase	LSM	LSM	LSM	LSM	SE
Phase 1	1.1	1.7	1.1	2.2	0.4
Phase 2	1.3	1.5	1.0	1.1	0.1

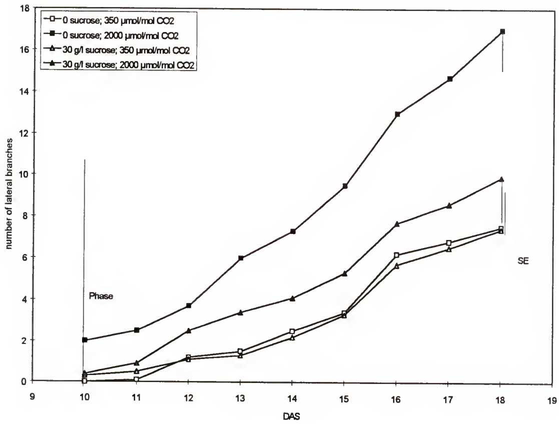

Figure 6-9. Number of lateral secondary branches from 10 to 18 DAS in lettuce seedlings grown with or without sucrose in the medium, with normal air (350 µmol mol^{-1} CO_2) or CO_2-enriched air (2000 µmol mol^{-1} CO_2).

Table 6-17. Least Squares Means (LSM) and relative Standard Errors (SE) of the slopes for the response of lettuce seedlings to 2 sucrose levels in the medium and 2 CO_2 concentrations in the air during the two phases of growth. Effects on lateral secondary branches. (Phase = 10 to 18 DAS).

	Sucrose (g l^{-1})				
	0		30		
	CO_2 (µmol mol^{-1})		CO_2 (µmol mol^{-1})		
	350	2000	350	2000	
Growth phase	LSM	LSM	LSM	LSM	SE
Phase	0.9	1.5	0.8	1.0	0.3

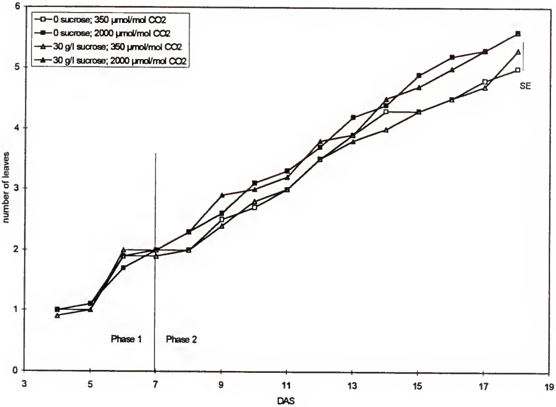

Figure 6-10. Number of differentiated leaves from 4 to 18 DAS in lettuce seedlings grown with or without sucrose in the medium, with normal air (350 µmol mol^{-1} CO_2) or CO_2-enriched air (2000 µmol mol^{-1} CO_2).

Table 6-18. Least Squares Means (LSM) and relative Standard Errors (SE) of the slopes for the response of lettuce seedlings to 2 sucrose levels in the medium and 2 CO_2 concentrations in the air during the two phases of growth. Effects on leaf number. (Phase 1 = 4 to 7 DAS; Phase 2 = 7 to 18 DAS).

	Sucrose ($g\ l^{-1}$)				
	0		30		
	CO_2 (µmol mol^{-1})		CO_2 (µmol mol^{-1})		
	350	2000	350	2000	
Growth phase	LSM	LSM	LSM	LSM	SE
Phase 1	0.2	0.2	0.2	0.2	0.0
Phase 2	0.2	0.2	0.2	0.2	0.0

10 DAS more leaves were produced by seedlings grown with 2000 µmol mol^{-1} CO_2 than by seedlings grown with 350 µmol mol^{-1} CO_2 (2.3 versus 2.0 ±0.1 SE, 8 DAS, 2.8 versus 2.4 ±0.3 SE, 9 DAS, 3.1 versus 2.7 ±0.2 SE, 10 DAS). After that, no differences in leaf number were detected between seedlings grown with 2000 and 350 µmol mol^{-1} CO_2 until 15, 16 and 17 DAS, at which time more leaves were again produced in seedlings grown with 2000 µmol mol^{-1} CO_2 (4.8 versus 4.3 ±0.3 SE, 15 DAS, 5.1 versus 4.5 ±0.5 SE, 16 DAS, 5.3 versus 4.7 ±0.5 SE, 17 DAS). At the end of the growth period, 18 DAS, leaf numbers were similar between treatments (Figure 6-10). Differentiation of new leaves during 18 days of lettuce seedling growth was not affected by sucrose (Tables 6-12 and 6-13).

Plant biomass 18 days after seeding

No effect on plant biomass was detected 18 DAS due to treatment (Table 6-19). Thus, exogenous sucrose application to the seedlings and CO_2 enrichment in the air had an effect on root branching, without affecting plant biomass. Sugar effects on plant growth differed when comparing exogenous versus endogenous paths of carbon delivery. Exogenous application of sucrose enhanced root growth only during the early days of seedlings growth, but after that exogenous sucrose suppressed root branching. Conversely, CO_2

Table 6-19. Means with significant probability for differences in the response of lettuce seedlings to sucrose feeding in the medium and CO_2 concentration in the air for dry mass, taproot length and root:shoot ratio 18 DAS.

Treatments		Dry Mass (mg)			Taproot Length (mm)	Root:Shoot Ratio
		Root	Shoot	Total		
CO_2 (μmol mol^{-1})	Sucrose (g l^{-1})					
350	0	4.2	47	51	100	0.09
350	30	4.9	38	43	78	0.12
2000	0	11.9	102	114	122	0.11
2000	30	11.1	81	92	88	0.13
Probability		0.8100	0.7738	0.7780	0.5996	0.7423
350		4.5	43	47	90	0.11
2000		11.5	91	103	105	0.12
Probability		0.0899	0.0856	0.0858	0.2223	0.1847
	0	8.0	74	82	111	0.10
	30	8.0	60	68	83	0.12
Probability		0.9829	0.4978	0.5477	0.0676	0.0559

enrichment enhanced lateral root branching during Phase 1, basal root branching from 4 to 18 DAS and secondary branch formation in nonsucrose-fed seedlings.

The differential morphological responses due to treatments, which occurred in lettuce seedling root growth, did not alter plant biomass, besides a greater shoot dry mass occurred 13 DAS in the first study (Table 6-10). This finding is important when screening of seedlings for extensive and differentiated root systems occur. Using CO_2 enrichment in controlled environment would be a valuable approach to increase early root branching in lettuce seedlings during greenhouse transplant production. The differentiated functions of basal and lateral roots have not yet been fully understood, but several authors have hypothesized that basal roots would provide a mean for plants to uptake surface-applied nutrients and water during crop production, and they may also play a role in supporting the plant (Bole, 1977; Eshel and Waisel, 1996; Jackson, 1995). Zobel (1996) reported that species that demonstrated the most drought tolerance had the most deeply penetrating root system, implying that plants with an extensive lateral root system would be favored in these instances.

Increased concentration of carbon dioxide in the atmosphere has received increased interest in recent years

(Eshel and Waisel, 1996). Plant adaptation to rising CO_2 concentrations in the atmosphere is a paramount priority these days, because it is estimated that CO_2 in the atmosphere will double from the 1978 average concentration of 338 µmol mol^{-1} by the middle or later part of the 21st century (Murray, 1995). Enrichment of atmospheric CO_2 has been reported to increase growth rate and yield of a wild variety of plants, and it is widely used in greenhouse crop production. Tremblay et al. (1987) increased growth rates of shoot and roots of celery transplants raised in greenhouse. Lettuce yield in a tunnel was enhanced by enrichment of CO_2 in the air (Enoch et al., 1970; Wittwer and Robb, 1963). Del Castillo et al. (1989) found that soybean plants grown under elevated CO_2 concentration had grater root mass than those grown under normal atmospheric CO_2 concentration. However, CO_2 concentration did not affect the rate of elongation of individual roots. The number of branches increased because of CO_2 enrichment, resulting in an increased total root length, without an increase of the volume of soil explored by the roots. Aguirrezabal et al. (1993) studied root systems in hydroponically grown sunflowers as affected by carbon nutrition. The results they obtained indicated that the control of carbon partitioning among various components of a single root system was determined by a combination of the distance of each sink from the source and its level of branching.

The present studies have indicated that differential responses in root growth of lettuce seedlings occurred when either exogenous sucrose or CO_2 enrichment in the atmosphere were supplied. The present research added another evidence in supporting the hypothesis that the path of carbon entry into a plant system can alter the plant growth itself differently. The finding that CO_2 enrichment enhanced root branch formation in lettuce seedlings, particularly basal root branches, could be further implemented to improve quality transplants produced in controlled environments.

Summary

Exogenous application of sucrose in Photo-Mixotrophic Whole Plant Culture systems is known to enhance plant growth. In Photosynthetic Whole Plant Culture systems exogenous sucrose application lead to altered sink-to-source status of leaves, because seedlings could use carbon supply through photosynthetic activity. Several lines of evidence support the hypothesis that the path of C entry into a plant system or organ can alter its effect at both the substrate and signaling levels. These alterations may be related to altered effects on growth and morphology. In the present studies, PWPC was used to address the hypotheses that lettuce root system can differentially respond to sucrose feeding and to free air flow, and further, that plant response to sucrose feeding might vary compared to carbon provided by CO_2 in the atmosphere.

Young lettuce seedlings cv. Valmaine were grown in bottles in a PWPC system for 13 days after seeding, either with or without 30 g l^{-1} of sucrose. Analysis of root architecture formation revealed that sucrose-fed seedlings produced more lateral, basal and total roots than nonsucrose-fed seedlings only during the early days of plant growth (from 4 to 7 DAS). After that, exogenous sucrose did not affect root branch formation. At 13 DAS root dry mass of lettuce seedlings was similar between treatments. Sucrose-fed seedlings had greater shoot dry mass than nonsucrose-fed seedlings.

In a second study, lettuce seedlings cv. Valmaine were grown in bottles in a PWPC system for 18 days. Plants were either supplied with or without 30 g l^{-1} of sucrose and with 350 µmol mol^{-1} or 2000 µmol mol^{-1} CO_2. Analysis of root architecture over 18 DAS revealed that CO_2 enrichment enhanced lateral root branching during Phase 1, basal root branching from 4 to 18 DAS and secondary branch formation in nonsucrose-fed seedlings. Exogenous application of sucrose enhanced root growth only during the early days of seedlings growth, but after that exogenous sucrose suppressed root branching. At 18 DAS seedling dry biomass did not differ among the treatments. Thus, exogenous sucrose application to the seedlings and CO_2 enrichment in the air had an effect on root branching, without affecting plant biomass.

The present study indicated that the differential morphological responses due to sucrose and CO_2 treatments, which occurred in lettuce seedling root growth, did not alter plant biomass. This finding is important when screening of seedlings for extensive root systems occur. Using CO_2 enrichment in controlled environment would be a valuable approach to increase early root branching in lettuce seedlings during greenhouse transplant production. The present studies have also demonstrated that differential responses in plants occurred when either exogenous sucrose or CO_2 enrichment in the atmosphere were supplied. The present research added another evidence in supporting the hypothesis that the path of carbon entry into a plant system can alter the plant growth itself differently.

CHAPTER 7

CARBON DIOXIDE ENRICHMENT IN THE AIR AND NITROGEN IN THE MEDIUM ALTER ROOT MORPHOLOGY, ARCHITECTURE AND GROWTH OF LETTUCE SEEDLINGS GROWN IN A PHOTOSHYNTHETIC WHOLE PLANT CULTURE (PWPC) SYSTEM

Introduction

The activities of carbon (C) and nitrogen (N) assimilatory processes are closely related to rates of plant growth and development. The constancy of this association has led modelers of physiological responses to explain whole-plant growth and coordinated growth between the shoot and root predominantly in terms of C and N interactions (Thornaley, 1976; Wann and Raper, 1979). Disruption in the supply of C or N can result in marked changes in the assimilation of the other. In the case of N stress, for example, two distinct changes in C utilization are commonly observed: a) starch accumulates in leaves (Ariovich and Cresswell, 1983; Radin and Eidenbock, 1986; Robinson and Baysdorfer, 1985; Wilson, 1975) and b) a larger portion of available carbohydrate is translocated from leaves into the root system, resulting in a increase of root:shoot ratio (Brouwer, 1962; Ingestad, 1979). The responses imply a general decline in carbohydrate utilization efficiency within the leaf canopy (Rufty et al., 1988). Increased translocation of carbohydrate to roots under N stress is probably related, at least in part, to a decreased rate of leaf canopy development (Rufty et al., 1988).

Changes in the relationships between C and N assimilatory processes can also occur when the C supply is increased. Doubling of the atmospheric CO_2 concentration, which is expected to occur by the middle of next century (Murray, 1995), is believed to increase crop yield (Sonnewald et al., 1996). Sonnewald et al. (1996) reported that the initial response to elevated CO_2 in the atmosphere can lead to an approximate increase of 50% in leaf photosynthesis. The increase in atmospheric CO_2 increases photosynthetic efficiency in terrestrial C_3 plants because the present CO_2 concentration is insufficient to saturate the ribulose-1,5-bisphosphate carboxylase/oxygenase enzyme system (Rubisco) and the increased CO_2 competitively inhibits ribulose-1,5-bisphosphate oxygenation and photorespiration (Long et al., 1996). However, an acclimation effect occurs, and the rate of photosynthesis again declines in many species, mainly due to a decline of Rubisco activity (Evans, 1983, 1987; Geiger and Servaites, 1991; Kramer, 1981; Sage et al., 1989; Sonnewald et al., 1996; Torisky and Servaites, 1984).

Long et al. (1996) hypothesized that if an increased atmospheric CO_2 concentration results in an increased net efficiency of photosynthetic CO_2 assimilation, then an increase in net photosynthetic C gain should be expected regardless of whether other requirements for plant growth, such as light and N, are limiting or saturating. This is because of an increased efficiency of light energy conversion into assimilated C rather than an increased supply of a co-limiting substrate.

Long et al. (1996) studied the long-term effect of CO_2 on stimulation of photosynthesis on ryegrass (*Lolium perenne*) grown in pots and in the field, with both low and high N supplies. An acclimatory loss of Rubisco activity and protein was found at both N levels, but more when N was limiting. Johnson et al. (1996) grew seedlings of two species of *Pinus* (*Pinus ponderosa* Laws. and *Pinus taeda* L.) with elevated CO_2 in the atmosphere and with or without N in the growing medium. These researchers reported (Johnson et al., 1996) that the N deficiency precluded plant response to CO_2 in *Pinus ponderosa* and reduced plant response on *Pinus taeda*. The elevated CO_2 and N levels caused a plant growth increase in the field for *Pinus ponderosa*, through an increase of root biomass. Unfortunately, no values of N and CO_2 concentrations were given, but the authors concluded that N can be a limiting factor for plant response to CO_2, because of plant increased N demand upon elevation of CO_2 availability.

Nitrate in sandy soils can be highly mobile, and it is efficiently absorbed at the root surface by the plant. The uptake of NO_3 remains largely unaffected by changes in its concentration in the soil solution around the roots until it is virtually depleted (Burns, 1980; Edwards and Barber, 1976). Plants tend to increase their NO_3 inflow rate (i.e. the rate of uptake per unit length of root) through the exposed roots, with up to a threefold increase observed for different crops when a highly localized NO_3 supply was imposed (Burns, 1991; Drew and Saker, 1975; Edwards and Barber, 1976; Robinson and Rorison, 1983). Drew et al. (1973) stated that plants increased root growth in the NO_3-rich zones, often at the expense of root growth in other regions. Since NO_3 inflow rates cannot be increased without limit when the proportion of roots exposed to NO_3 is reduced, Burns (1991) suggested that N uptake must become dependent on root length when the number of absorbing roots is small, and that the minimum amount of roots required to sustain N uptake will depend on the N demand of the plant. Imsande and Touraine (1994) stated that to cope with variations in mineral concentrations in soil, plants have evolved mechanisms to regulate the activity of the uptake system in such a way that the net intake of a nutrient depends on the plant need for the given element rather than its concentration in the medium.

Nitrate uptake is of special interest in mineral nutrition since NO_3 is absorbed at a relatively high rate by the plant (Imsande and Touraine, 1994). However, while there are many studies on the effects of N stress on plant growth, very few have focused on the effect of abundant availability of N on plant growth. To our knowledge, the effects of N availability on lettuce seedling root morphology and architecture have not been reported. Soil root distribution differences between plant species can provide clues regarding spatial and temporal differences in the uptake of water and nutrients (Klepper, 1987; Parris and Bazzaz, 1976).

Measurements of root biomass and root length are typically used to describe root distribution in the soil profile, but analysis of root morphology and architecture can improve the understanding of water and nutrient extraction from soil (Fitter, 1985). A major difficulty in studying root systems in the soil is the extraction of fine roots and measurement of architectural variables. Fitter and Stickland (1991) and Lynch and van Beem (1993) suggested that the architecture of a root system may have ecological implications for uptake of water and nutrients from soil. Fitter (1986, 1987) suggested that, in general, plants with a more herringbone-like distribution of roots, that is, with branches mainly along the central root axis, may occur under low soil resource availability, whereas secondary branches increase when resources are present in abundant supply, thereby increasing acquisition of water and nutrients.

The effect of elevated CO_2 in the atmosphere has often been studied by scientists, but mostly as related to shoot growth. Because changes of CO_2 concentration in the air affect photosynthesis rate, and because N supply might become a limiting factor during an increase in photosynthesis rate, increasing the N supply in the root system may or may not increase net plant growth. More importantly, an increase in N supply in the rooting medium could change root morphology and architecture to allow the roots to adapt to the changed conditions. To address these hypotheses a Photosynthetic Whole Plant Culture (PWPC) system (Chapter 4) was used to grow lettuce, allowing direct observation of root morphology and architecture of young lettuce seedlings. In the present research PWPC was used to test: 1) whether CO_2 enrichment results in a translocation of carbohydrate to roots, affecting root architecture and development; 2) whether increased N levels in the growth medium causes adaptive changes in the roots leading to changes in root architecture and development.

Materials and Methods

Gelled medium 'Phytagel' (Sigma Chemical Co., St. Louis, MO) (2.5 g l^{-1}) was used as a substrate for growing and evaluating root morphology and development of lettuce seedlings from radicle protrusion to 18 days after seeding (DAS). The basal nutrient medium contained inorganic salts according to Murashige and Skoog (1962) (Table 7-1), with a total of 60 mM of N from NH_4NO_3 (41.2 mM N) and KNO_3 (18.8 mM N). A second level of N was introduced by adding 60 mM as NH_4NO_3 for a total of 120 mM N. Solution pH was adjusted to 6.5 with 1 N NaOH prior to autoclaving. Forty glass 1-l bottles (Pyrex, Corning, NY) were prepared by filling each with 650 ml of medium and autoclaving them for 35 min at 130° C and 1.1 kg cm^{-2} (15 psi). After autoclaving, the medium was cooled to solidify at ambient temperature. Another 50-ml layer of medium was poured over this, which contained 3 g l^{-1} of activated charcoal (Grand Island Biological Company, Grand Island, NY) to reduce light in the root zone and avoid Fe-catalyzed Photooxidation of EDTA

Table 7-1. Composition of Murashige and Skoog (1962) medium for tissue culture.

Major elements				Minor elements		
Salts	mg/l		mM	Salts	mg/l	µM
NH_4NO_3	1650	N	41.20	H_3BO_3	6.2	100
KNO_3	1900	N,K	18.80	$MnSO_4 \cdot 4H_2O$	22.3	100
$CaCl_2 \cdot 2H_2O$	440	Ca	3.00	$ZnSO_4 \cdot 4H_2O$	8.6	30.0
$MgSO_4 \cdot 7H_2O$	370	Mg,S	1.50	KI	0.83	5.0
KH_2PO_4	170	P,K	1.25	$Na_2MoO_4 \cdot 2H_2O$	0.25	1.0
Na_2EDTA	37.3	Na	0.20	$CuSO_4 \cdot 5H_2O$	0.025	0.1
$FeSO_4 \cdot 7H_2O$	27.8	Fe	0.10	$CoCL_2 \cdot 6H_2O$	0.025	0.1
Salts added for 120 mM total N						
NH_4NO_3	2402	N	60.00			

present in the substrate (Hangarter and Stasinopoulos, 1991).

Seeds of lettuce (*Lactuca sativa* L.) cv. Valmaine were surface-sterilized by soaking them in a solution of 2% NaOCl and 0.1% Tween 20 for 20 minutes. The seeds were then rinsed five times in sterilized-deionized water, placed in a Petri dish with filter paper no.3 (Whatman International Ltd. Maidstone, England), and moistened with sterilized-deionized water. The seeds were germinated for 24 hr at 20-22°C under continuous fluorescent light (Osram Sylvania Inc., Danvers, MA, 50 $\mu mol \cdot m^{-2}s^{-1}$ PPF). When root length reached 2 mm, after 24 hr, one seedling was transferred in each bottle by placing it at the center top surface of the gelled medium. The seedling was carefully inserted into the medium leaving the upper hypocotyl above the gel surface. Glass bottles were externally covered with aluminum foil around the medium to block light from reaching the root surface. Forty bottles were placed in a growth room on a bench as previously described (Chapter 4). The total PPF was 300 $\mu mol \cdot m^{-2}s^{-1}$ and the photoperiod was 16hr. Temperature in the room was kept constant at 23°C to maintain internal bottle temperature of 28/23°C ± 1°C (day/night). Air flow through the bottles was maintained as previously described (Chapter 4). Two concentrations of CO_2 in the air were used, normal air CO_2

concentration (350 µmol mol^{-1} CO_2) and CO_2-enriched air (normal air plus CO_2 up to a total of 2000 µmol mol^{-1} CO_2).

Numbers of lateral and basal roots and differentiated leaves were counted daily for 18 (DAS), by visual observation through the bottle. Total root numbers (lateral + basal roots) were computed daily. Secondary branches emerging from the lateral roots were counted starting from initiation of the first branch root (9 DAS) until 14 DAS, by direct observations through the bottle. At harvest, root and shoot dry weights were obtained after oven drying of each seedling for 96 hr at 60°C. Taproot length was measured for each plant with a ruler with an approximation of 1 mm.

Treatments consisted of 2 levels of N (60 and 120 mM) (Table 7-1) and 2 concentrations of CO_2 (350 and 2000 µmol mol^{-1}). The experimental design was a split-plot design with 2 blocks, N as sub-plot and CO_2 as main plot treatment. Five observations per block were used. Data were analyzed using a SAS statistical system (SAS Institute Inc., 1989). All data taken daily were submitted to the analysis of variance of repeated measurements to investigate the plant growth response over time (Littell, 1989; Chapter 8). All χ^2 tests of Mauchly's criterion were highly significant and the G-G and H-F epsilons were lower than 1 (Appendix A8-19), consequently the G-G adjusted P values were used (Greenhouse and Geisser, 1959) for all interactions with time. Data of the growth trend were plotted over time for each treatment. Trend analyses were performed by considering three main growth phases: 1) phase of initial growth, from the protrusion of lateral and/or basal roots (4 DAS to 5 DAS); 2) phase of expected response to treatments (5 to 7 DAS); 3) phase of continuation of growth (7 to 18 DAS). In the case of lateral branches only 1 phase was considered, from the initiation of branches (9 DAS to 14 DAS). In Phases 2 and 3 slopes of the line segments were computed and analyzed. Data taken at the termination of the experiment were submitted to the analysis of variance. Least Squares Means (LSM) and Standard Errors (SE) were calculated for each measured variable.

Results and Discussion

Validity of the F test for the main effects of N and CO_2 and their interaction did not require the Huynh-Feldt (1970) condition of the repeated measurements (Littell, 1989; Chapter 8), consequently the univariate F test was considered correct (Tables 7-2 and 7-3). Carbon dioxide and N levels had an independent effect on seedling growth. Enriching CO_2 to 2000 µmol mol^{-1} did not affect the overall means of each measured variables. Overall means of lateral and total root numbers were increased by 60 mM N in the medium compared to 120 mM N. The mean lateral root numbers were 18.2 in seedlings grown with 60 mM N and 13.7 with 120 mM N (P=0.0087). The mean total root numbers were 26.4 with 60 mM N and 18.6 with 120 mM N (P=0.0208) (Appendix C-1).

Interaction between time and N in the medium was significant for root architecture and the total number of leaves (Tables 7-2 and 7-3). Interaction between time and CO_2 was significant for basal and total root number and the number of lateral branches. Interaction among time, N and CO_2 was significant in the case of total root number and the total number of leaves.

Cotyledons of all seedlings were already fully expanded when first lateral and basal roots began to emerge from the radicle (Figure 7-1). This phenomenon occurred in any of the previous studies confirming that in lettuce seedlings primary branch differentiation does not occur prior to cotyledon unfolding, as reported in bell pepper seedlings (Chapters 3 and 6; Stoffella et al., 1988).

The number of lateral roots was affected by an interaction between time and the level of N supplied in the growth medium (Table 7-2). More lateral roots emerged from the radicle when less N was supplied, regardless of the amount of CO_2 applied. This would suggest that the greater

Table 7-2. Univariate analysis of variance and G-G adjustments of repeated measures for lateral, basal and total root number of lettuce seedlings from 4 to 18 DAS.

		Root number					
		Lateral		Basal		Total	
Source of variation	DF	Univariate F Test	G - G Adjust.	Univariate F Test	G - G Adjust.	Univariate F Test	G - G Adjust.
Block	1						
CO_2	1	0.1421		0.1644		0.1495	
CO_2 * Block (error a)	1						
N	1	0.0087		0.0757		0.0208	
CO_2 * N	1	0.0742		0.2145		0.1099	
N * Block (CO_2) (error b)	2						
Sampling error	16						
Analysis over time							
Time	14	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Time * Block	14	0.2576	0.3060	0.0002	0.0303	0.0109	0.0809
Time * CO_2	14	0.0043	0.0519	0.0001	0.0119	0.0001	0.0001
Time * CO_2 *Block	14	0.0458	0.1371	0.8882	0.6466	0.0019	0.0420
Time * N	14	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Time * CO_2 * N	14	0.1574	0.2396	0.0015	0.0572	0.0002	0.0182
Error	252						

Table 7-3. Univariate analysis of variance and G-G adjustments of repeated measures for number of leaves and lateral secondary branches of lettuce seedlings from 4 to 18 DAS.

		Number of				
		Leaves			Secondary Branches	
Source of variation	DF	Univariate F Test	G - G Adjustment		Univariate F Test	G - G Adjustment
Block	1					
CO_2	1	0.5682			0.1403	
CO_2 * Block (error a)	1					
N	1	0.2239			0.2027	
CO_2 * N	1	0.2372			0.7110	
N * Block (CO_2) (error b)	2					
Sampling error	16					
Analysis over time				DF		
Time	14	0.0001	0.0001	5	0.0001	0.0001
Time * Block	14	0.0004	0.0272	5	0.9147	0.6932
Time * CO_2	14	0.0457	0.1469	5	0.0027	0.0395
Time * CO_2 *Block	14	0.0887	0.1920	5	0.7964	0.5822
Time * N	14	0.0001	0.0089	5	0.0040	0.0456
Time * CO_2 * N	14	0.0001	0.0059	5	0.5818	0.4473
Error	252			90		

concentration of N in the medium depressed lateral root formation.

Lateral roots began to emerge 4 DAS (Figure 7-2). Statistical analyses of the phases of growth on the differentiation of lateral roots indicated that lateral roots rapidly increased in number between 4 and 5 DAS, with no differences among treatments (Tables 7-4 and 7-5). From 5 to 7 DAS lateral root number increased very rapidly. Seedlings grown with 120 mM N and 2000 µmol mol^{-1} CO_2 had a constant increase in the formation of lateral roots, with a greater estimated slope (3.0) than seedlings growing with 350 µmol mol^{-1} CO_2 (1.9 with 60 mM N and 1.6 with 120 mM N).

In the third phase of growth, lateral root numbers continued to increase, but with a smaller slope than in the second phase. The trend of lateral root number differentiation of seedlings grown with 120 mM N in the medium was lower than the trend with 60 mM. Eighteen DAS an interaction effect on lateral root number between N and CO_2 was not detected. Seedlings grown with less N had more lateral roots than seedlings grown with more N (28.9 versus 22.6, P=0.0001). Seedlings grown with 2000 µmol mol^{-1} CO_2 had more lateral roots than seedlings grown with 350 µmol mol^{-1} CO_2 (28.4 versus 23.1, P=0.0005). The mean number of lateral roots 18 DAS (26.0 ±0.6 SE) was greater than in an other study in which plants were grown for 18 days (18.3 ±0.6 SE) (Chapter 6). The major difference between the two studies was the photosynthetic photon flux density. In the previous study, PPF was 250 µmol $m^{-2}s^{-1}$, whereas in the present study PPF was 300 µmol $m^{-2}s^{-1}$. An overall increase in root differentiation might have occurred due to the increased light intensity.

Number of basal roots was affected by an interaction between time and level of N supplied in the growing medium (Table 7-2). More basal roots emerged from the radicle when less N was supplied, regardless of the amount of CO_2 in the air. This suggested that a concentration of 120 mM of N in the medium depressed basal root emergence, as it did for lateral roots. Conversely, the interaction time by CO_2 affected basal root number. When seedlings were supplied with 2000 µmol mol^{-1} CO_2, more basal roots emerged from the radicle, than when seedlings were supplied with 350 µmol mol^{-1} CO_2.

In a previous study (Chapter 6), an interaction effect of time x CO_2 concentration on basal root number was detected in seedlings grown 4 to 18 DAS with 350 or 2000 µmol mol^{-1} CO_2. The elevated CO_2 concentration led to an increase in basal root number, as in the present study. Also, lateral root number was not affected by the

Table 7-4. P values of the different segments of the trend analysis of lateral, basal and total root number, number of leaves and lateral secondary branches. (Phase 1 = 4 to 5 DAS; Phase 2 = 5 to 7 DAS, slope; Phase 3 = 7 to 18 DAS, slope; Phase = 9 to 14 DAS, slope).

	Lateral Roots			Basal Roots		
Treatments	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
N	0.3642	0.8782	0.0065	0.4233	0.2245	0.0977
CO_2	0.3961	0.2028	0.4824	0.2662	0.3692	0.1994
CO_2 * N	0.6025	0.7145	0.3129	0.2729	0.9701	0.1844

	Total Roots			Number of Leaves		
Treatments	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
N	0.3244	0.5470	0.0198	0.3073	0.3073	0.2147
CO_2	0.4224	0.0116	0.3309	0.7952	0.7952	0.6550
CO_2 * N	0.4561	0.6716	0.2170	0.6951	0.6951	0.1790

	Secondary Branches
Treatments	Phase
N	0.1099
CO_2	0.0896
CO_2 * N	0.3657

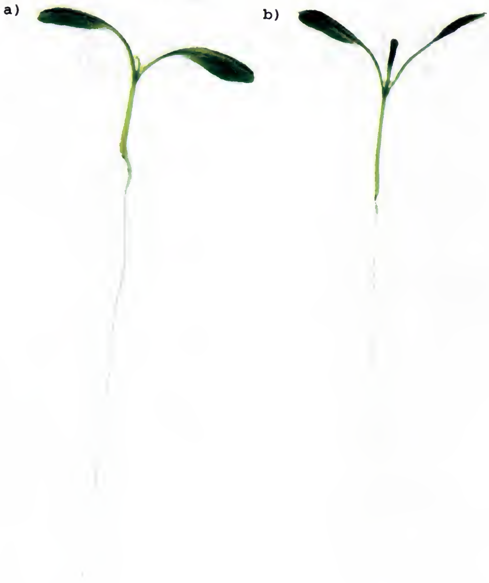

Figure 7-1. Lettuce seedlings cv. Valmaine 4 DAS (a) and 5 DAS (b), grown with 60 mM N and 350 µmol mol CO . Cotyledons were full expanded 4 DAS and when the first basal root emerged from the radicle.

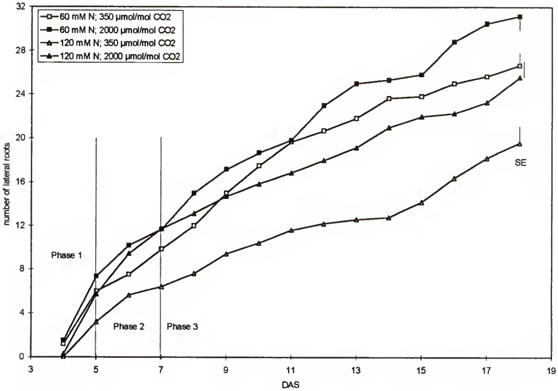

Figure 7-2. Number of lateral roots in lettuce seedlings from 4 to 18 DAS.

Table 7-5. Least Squares Means (LSM) and relative Standard Errors (SE) for the response of lettuce seedlings to 2 N levels in the medium and 2 CO_2 concentrations in the air during the three phases of growth. Effects on lateral root number. (Phase 1 = 4 to 5 DAS, difference of the values; Phase 2 = 5 to 7 DAS, slopes; Phase 3 = 7 to 18 DAS, slopes).

	N (mM)							
	60				120			
	CO_2 (μmol mol^{-1})				CO_2 (μmol mol^{-1})			
	350		2000		350		2000	
Growth phase	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Phase 1	4.9	1.0	5.8	1.0	3.2	1.1	5.3	1.0
Phase 2	1.9	0.5	2.2	0.5	1.6	0.5	3.0	0.5
Phase 3	0.7	0.1	0.9	0.1	0.5	0.1	0.6	0.1

interaction of time x CO_2 concentration.Initial response (prior to the acclimation effect) to elevated CO_2 levels is an increased photosynthesis rates (Jang and Sheen, 1996 in co2 meeting; Long et al., 1996; Sonnewald et al., 1996). Increased carbon allocation to roots due to elevated CO_2 concentrations has been reported in different species (Norby, 1994; Rogers et al., 1994; Stulen and den Hertog, 1993in hungate 96, co2 meeting). However, a distribution of the C allocation between different types of roots, i.e. basal versus lateral roots, to our knowledge has not been reported. Basal and lateral roots have been reported to be under different genetic control in tomato (Zobel, 1975). A differential response between basal and lateral root formation in lettuce seedlings to elevated CO_2 concentration found in the previous and the present study might be hypothesized as a consequence of different C allocation programs between basal and lateral roots. Nitrogen availability was not a limiting factor for lettuce seedlings in differentiating primary branches. On the contrary, increased levels of N without increased CO_2 availability depressed lateral and basal root formation from 4 to 18 DAS.

Basal roots emerged from the radicle of lettuce seedlings 4 DAS (Figure 7-3). Statistical analyses of the phases of growth on the differentiation of basal roots indicated that, conversely to lateral roots, basal root number did not increase rapidly during Phase 1 (from 4 to 5 DAS) (Tables 7-4 and 7-6). During the first phase of growth, basal root number did not differ among the treatments, as it did for lateral root number. In a series of studies with the same growth system (Chapter 6), a similar pattern was observed with seedlings which were either sucrose-fed or not and supplied with either 350 or 2000 µmol mol^{-1} CO_2. The present study confirmed that basal roots generally emerged prior to lateral roots or at the same time in any given 'Valmaine' lettuce seedling, but that lateral roots increased in number more rapidly during the 4 to 5 DAS period. During the early stages of growth, lettuce seedlings used the food reserves in the seeds, and N and CO_2 concentration did not appear to alter this pattern.

Basal root number increased more rapidly in Phase 2 than in Phase 1, without any differential response to the treatments (Table 7-6). During Phase 3, seedlings grown with 120 mM N in the medium and supplied with normal air tended to differentiate fewer basal roots than the other seedlings (Table 7-6). The main effect of CO_2 and the N by CO_2 interaction effect on basal root number were not significant for plants at 18 DAS. More basal roots emerged in seedlings grown with 60 mM N than seedlings grown with 120 mM (16.5

versus 10.6, P=0.0464). Seedlings grown with 120 mM N and 350 µmol mol^{-1} CO_2 had the fewest basal roots at 18 DAS.

During the 18 DAS growing period an interaction between time, N in the medium and CO_2 concentration in the atmosphere affected the total root number, but not lateral and basal root numbers (Table 7-2). When seedlings were grown with 60 mM N in the medium, more roots formed (Figure 7-4) compared to seedlings grown with 120 mM N and 2000 µmol mol^{-1} CO_2. When seedlings were grown with 120 mM N, there were more roots with 2000 µmol mol^{-1} CO_2, compared to 350 µmol mol^{-1} CO_2. The concentration of CO_2 affected total root development in relation to N level. When more N was provided, seedlings responded by producing more roots over time only when CO_2 concentration in the air were increased. However, when less N was present in the medium, more roots were produced regardless of CO_2 enrichment.

Statistical analyses of the phases of growth on the differentiation of total roots indicated that total root number increased rapidly during Phase 1 (Tables 7-4 and 7-7, Figure 7-4), mainly due to rapid lateral root differentiation. During Phase 2, more roots emerged from seedlings grown with 2000 µmol mol^{-1} CO_2 than from seedlings grown with 350 µmol mol^{-1} CO_2. CO_2 had no effect on lateral or basal root number during Phase 2. However, the overall

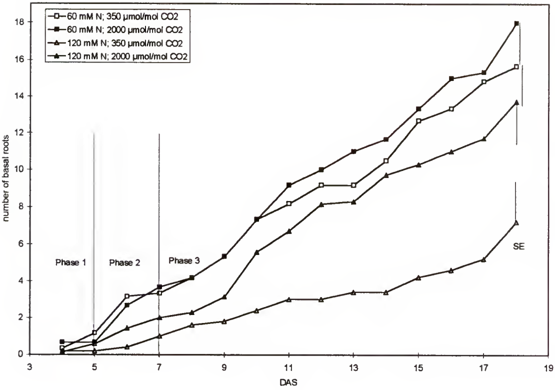

Figure 7-3. Number of basal roots in lettuce seedlings from 4 to 18 DAS.

Table 7-6. Least Squares Means (LSM) and relative Standard Errors (SE) for the response of lettuce seedlings to 2 N levels in the medium and 2 CO_2 concentrations in the air during the three phases of growth. Effects on basal root number. (Phase 1 = 4 to 5 DAS, difference of the values; Phase 2 = 5 to 7 DAS, slopes; Phase 3 = 7 to 18 DAS, slopes).

	N (mM)							
	60				120			
	CO_2 (μmol mol^{-1})				CO_2 (μmol mol^{-1})			
	350		2000		350		2000	
Growth phase	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Phase 1	0.8	0.4	0.0	0.4	0.0	0.4	0.4	0.3
Phase 2	1.1	0.3	1.5	0.3	0.5	0.3	0.8	0.3
Phase 3	0.6	0.1	0.6	0.1	0.2	0.1	0.5	0.1

primary root differentiation (i.e. total root number) was enhanced during Phase 2 when CO_2 was enriched in the atmosphere. The same effect was observed in a previous study from 4 to 7 DAS (Chapter 6). During Phase 3, CO_2 enrichment in the air did not change the trend of total root differentiation, as in the case of lateral and basal root differentiation. A N effect occurred on the number of total roots formed. In fact, seedlings grown with less N in the medium differentiated more total roots than seedlings grown with more N in the medium. Trends in basal root number differentiation did not change during Phase 3 (Table 7-4) due to N concentration in the medium, whereas trends of lateral root number did. Consequently, the trend differences observed in total root formation during Phase 3 can be attributed to the trend differences in lateral root formation.

Lateral secondary branches were counted from the first day of their emergence (9 DAS) through 14 DAS. When the number of branch roots reached 20 they became difficult to count in the bottle growth system. Because of this, branches were not counted after 14 DAS. Some branches began to emerge 9 DAS in seedlings growing with 60 mM N, but most seedlings began to form branches 10 DAS. The number of secondary branches was affected by an interaction between CO_2

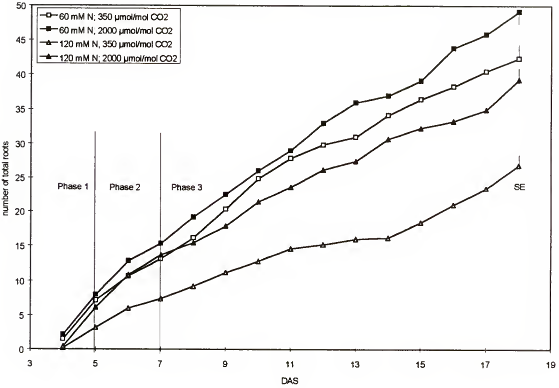

Figure 7-4. Number of total roots in lettuce seedlings from 4 to 18 DAS.

Table 7-7. Least Squares Means (LSM) and relative Standard Errors (SE) for the response of lettuce seedlings to 2 N levels in the medium and 2 CO_2 concentrations in the air during the three phases of growth. Effects on total root number. (Phase 1 = 4 to 5 DAS, difference of the values; Phase 2 = 5 to 7 DAS, slopes; Phase 3 = 7 to 18 DAS, slopes).

	N (mM)							
	60				120			
	CO_2 (µmol mol^{-1})				CO_2 (µmol mol^{-1})			
	350		2000		350		2000	
Growth phase	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Phase 1	5.9	1.1	5.8	1.1	3.0	1.2	5.4	1.1
Phase 2	3.0	0.4	3.7	0.4	2.1	0.5	3.8	0.4
Phase 3	1.3	0.1	1.5	0.1	0.8	0.1	1.1	0.1

concentration and time. Seedlings grown with 2000 µmol mol^{-1} CO_2 produced more secondary branches from 9 to 14 DAS than seedlings grown with 350 µmol mol^{-1} CO_2 (Figure 7-5). Branch root development from 10 to 18 DAS was also increased with 2000 µmol mol^{-1} CO_2 in a previous study (Chapter 6). The N levels interacted with time. The 60 mM N concentration enhanced secondary branch formation from 9 to 14 DAS. Branch root development seemed very similar to the pattern of lateral and basal root development. At 120 mM N, seedlings formed fewer branches, either primary (lateral and basal roots) or secondary (lateral branches).

Estimated linear slopes for secondary branch formation did not differ between treatments (Table 7-4), probably because the pattern of branch formation was not linear during the growth period. At 14 DAS, 16.4 secondary branches were formed in seedlings grown with 2000 µmol mol^{-1} CO_2 and 7.5 secondary branches were formed in seedlings grown with 350 µmol mol^{-1} CO_2 (P=0.0113). At 14 DAS 8.6 secondary branches were formed in seedlings grown with 120 mM N and 15.3 secondary branches were formed in seedlings grown with 60 mM N (P=0.0447).

A significant interaction on leaf differentiation occurred among N level in the medium, CO_2 concentration in the air and time (Table 7-3, Figure 7-6). From 9 to 14 DAS,

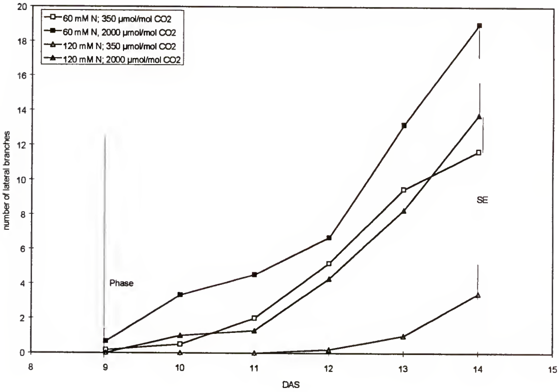

Figure 7-5. Number of lateral secondary branches in lettuce seedlings from 9 to 14 DAS.

Table 7-8. Least Squares Means (LSM) and relative Standard Errors (SE) for the response of lettuce seedlings to 2 N levels in the medium and 2 CO_2 concentrations in the air during the Phase 3 of growth. Effects on number of lateral secondary branches. (Phase = 9 to 14 DAS, slopes).

	N (mM)							
	60				120			
	CO_2 (µmol mol^{-1})				CO_2 (µmol mol^{-1})			
	350		2000		350		2000	
Growth phase	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Phase	1.2	0.3	1.8	0.3	0.3	0.3	1.3	0.3

fewer leaves were differentiated in seedlings grown with 120 mM N and 350 µmol mol^{-1} CO_2, than in seedlings grown with 120 mM N and 2000 µmol mol^{-1} CO_2 or with 60 mM N and either 350 µmol mol^{-1} or 2000 µmol mol^{-1} CO_2. The first true leaf appeared 4 DAS in all seedlings. From 4 to 5 DAS, the number of leaves remained similar, and leaf differentiation differences among the treatments during the 3 phases were not observed (Table 7-4). Eighteen DAS, 4.9 leaves formed in seedlings grown with 120 mM N and 350 µmol mol^{-1} CO_2, and 6.2 in seedlings grown with 120 mM N and 2000 µmol mol^{-1} CO_2, number of leaves were 6.6 in seedlings grown with 60 mM N and 350 µmol mol^{-1} CO_2, and number of leaves were 6.5 in seedlings grown with 60 mM N and 2000 µmol mol^{-1} CO_2.

The effects of CO_2 and N concentrations affected leaf differentiation in a similar fashion as they affected root architecture. Lettuce seedlings grown for 18 DAS were able to increase primary and secondary root branch formation and leaf number when 60 mM N was supplied, regardless of CO_2 availability. However, when more N was supplied, i.e. 120 mM, branch development and leaf formation were depressed without CO_2 enrichment.

Plant biomass measured at the end of the experiment in terms of root, shoot and total dry weights, did not differ among the treatments (Table 7-10). Taproot length was also

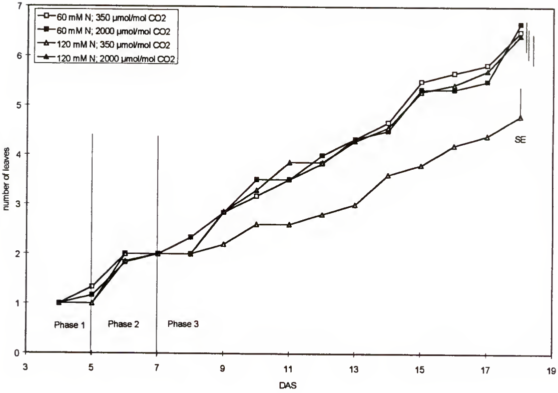

Figure 7-6. Number of differentiated leaves in lettuce seedlings from 4 to 18 DAS.

Table 7-9. Least Squares Means (LSM) and relative Standard Errors (SE) for the response of lettuce seedlings to 2 N levels in the medium and 2 CO_2 concentrations in the air during the three phases of growth. Effects on number of differentiated leaves. (Phase 1 = 4 to 5 DAS, difference of the values; Phase 2 = 5 to 7 DAS, slopes; Phase 3 = 7 to 18 DAS, slopes).

	N (mM)							
	60				120			
	CO_2 (μmol mol^{-1})				CO_2 (μmol mol^{-1})			
	350		2000		350		2000	
Growth phase	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Phase 1	0.3	0.13	0.2	0.13	0.0	0.15	0.0	0.12
Phase 2	0.3	0.07	0.4	0.07	0.5	0.07	0.5	0.06
Phase 3	0.2	0.02	0.2	0.02	0.1	0.03	0.2	0.02

Table 7-10. Means with significant probability for differences in response of lettuce seedlings to 2 N levels and 2 CO_2 concentrations for dry mass, root to shoot ratio and taproot length 18 DAS.

Treatments		Dry Mass (mg)			Root:Shoot	Taproot Length (mm)
CO_2 (μmol mol^{-1})	N (mM)	Root	Shoot	Total		
350	60	10	129	139	0.08	100
2000	60	14	145	159	0.10	116
350	120	2	34	36	0.07	102
2000	120	12	134	145	0.09	110
Probability		0.5067	0.3167	0.3292	0.5968	0.5537
350		6	81	87	0.07	101
2000		13	139	152	0.09	113
Probability		0.2888	0.3164	0.3142	0.0129	0.1496
	60	12	137	149	0.09	108
	120	7	84	91	0.08	106
Probability		0.2823	0.2625	0.2644	0.2258	0.7670

similar among the treatments. Root to shoot ratio increased in seedlings grown with 2000 µmol mol^{-1} CO_2 compared to seedlings grown with 350 µmol mol^{-1} CO_2.

Environmental conditions such as levels of N in the medium and concentrations of CO_2 in the air affected root architecture of seedlings. Partitioning between root and shoot was altered. In a previous study (Chapters 3) exogenous sucrose application increased shoot, root, total and dry mass of different genotypes of lettuce seedlings grown for 9 DAS. However, the plant culture system was different and exogenous sucrose application has been shown to enhance the early growth of seedlings (Chapter 6). In a previous study, using the Photosynthetic Whole Plant Culture system, 'Valmaine' lettuce seedlings did not differ in root dry mass when grown with or without exogenous sucrose for 13 DAS (Chapter 6). Shoot dry mass was enhanced because of sucrose application.

The possibility of lettuce seedlings to use CO_2 in the atmosphere as a C source was previously assessed (Chapter 6). Because different treatments were involved, complete comparison cannot be made. Nevertheless, a decreased response to CO_2 enrichment in the atmosphere of seedlings in terms of plant biomass was detected, and it would be confirmed by the present study. This could be due to an acclimation effect of the plants to elevated CO_2 concentration (Sonnewald et al., 1996). However, the effect of elevated CO_2 in the atmosphere has been generally related by scientists to shoot growth. The previously reported and the present studies demonstrated that increased CO_2 concentration in the atmosphere changed lettuce seedlings root architecture, by enhancing root branch formation. The architecture of a root system is important for plant growth and survival, because an elaborated branch formation in the root system increases the surface area available for water and mineral uptake (Fitter, 1986, 1987; MacIsaac et al., 1989). In addition, a primary root system which presents increased branching provides more support of the shoot to the soil.

The effects of N availability in plant growth are usually reported in terms of plant biomass. Elevated N levels in a standard greenhouse growth medium increased plant N concentration in tomato seedlings, with a greater N increase in the leaves than in the roots (Nicola et al., 1995). The root to shoot ratio decreased when more N was applied. A decrease in root to shoot ratio in lettuce seedlings growing with elevated N levels in a commercial greenhouse growth medium has been documented (Nicola and Cantliffe, 1996). The present study demonstrated that N availability did not change plant biomass and assimilate partitioning in lettuce seedlings. However, root architecture was affected by N. Increased N in the growth medium to 120 mM led to plants that produced fewer roots, particularly when the plants were grown with 350 µmol mol^{-1} CO_2. Nitrogen availability did not limit primary root branch formation. On the contrary, increased levels of N (120 mM) without increased CO_2 availability (350 µmol mol^{-1}) depressed lateral and basal root formation.

The information that increased N concentration in the growth medium alters root morphology and development can be extremely useful to researchers and growers studying and/or growing young seedlings. Enhanced early root growth is desirable during greenhouse transplant production to increase seedling field stand establishment and subsequent yields. Consequently, reducing N application in the growth medium may be a valuable tool to regulate and enhance root growth and development in lettuce seedlings. In addition, enriching the atmospheric CO_2 in controlled environments would be a valuable approach to increase early root branch formation.

Summary

The effect of elevated CO_2 in the atmosphere has often been studied by scientists, but generally as related to shoot growth. Because changes of CO_2 concentration in the air affect photosynthesis rate, and because N supply might become a limiting factor during an increasing photosynthesis rate, increasing the N supply in the root system may or may not increase net plant growth. More importantly, an increase in N supply in the rooting medium could change root morphology and architecture to allow the roots to adapt to the changed conditions. In the present research a Photosynthetic Whole Plant Culture system was used to test: 1) whether CO_2 enrichment causes translocation of carbohydrate to roots, affecting root architecture and development; 2) whether increased availability of N in the growth medium causes adaptive changes in the roots affecting root architecture and development.

'Valmaine' lettuce seedlings were grown in bottles for 18 days after seeding (DAS). Plants were supplied of either 60 mM or 120 mM of N as NH_4NO_3 and KNO_3 in the medium. Two concentrations of CO_2 were applied, 350 µmol mol^{-1} CO_2 (standard atmospheric air), or 2000 µmol mol^{-1} CO_2 (CO_2-enriched air). At 2000 µmol mol^{-1} CO_2 root growth was enhanced in terms of basal and total root numbers and

secondary root branches, compared to 350 µmol mol^{-1} CO_2. With 60 mM N in the medium seedlings were able to form more lateral, basal and total roots over time than with 120 mM N. Number of leaves was enhanced by 2000 µmol mol^{-1} CO_2, regardless of N concentration in the medium, and by 350 µmol mol^{-1} CO_2 when 60 mM N were present in the medium, compared to the number of leaves differentiated in seedlings growing with 120 mM N in the medium and 350 µmol mol^{-1} CO_2.

The early phase of root and leaf formation (4 to 5 DAS) was not affected by any of the treatments. Lateral and total root number increased rapidly during this early phase in all seedlings. Basal roots, even though emerging from the radicle prior to the lateral roots, increased more rapidly at a later stage of seedling growth (5-6 DAS). Root, shoot and total dry weights at harvest did not differ among the treatments. Root to shoot ratio increased in seedlings grown with 2000 µmol mol^{-1} CO_2 compared to seedlings grown with 350 µmol mol^{-1} CO_2. Environmental conditions such as levels of N in the medium and concentrations of CO_2 in the air affected root architecture of seedlings without changing plant biomass. However, photosynthate partitioning between root and shoot was altered.

The present studies demonstrated that increased CO_2 concentration in the atmosphere changed lettuce seedling root architecture, by enhancing branch formation. The study also demonstrated that N availability did not change plant biomass and partitioning in lettuce seedlings. However, root architecture was affected by N. The increased 120 mM level of N in the growing medium led to plants which produced fewer roots, particularly when growing in 350 µmol mol^{-1} CO_2. If this information would be confirmed in soil-grown seedlings, improved root branch systems of lettuce seedlings might be achieved by reducing N application in the soil.

CHAPTER 8
STATISTICAL ANALYSIS OF REPEATED MEASUREMENTS TO INVESTIGATE ROOT GROWTH DEVELOPMENT

Introduction

Plant growth trends over time are often investigated to study the response of a plant or a system to various treatments. Morphological variables can be measured at several points in time on the same plant. Graphical plots of the collected data can then be constructed over time and can reveal growth patterns of the plant. The split-plot in time design, with time as a subplot factor, has often been used to analyze the response over time to treatments (Gomez and Gomez, 1984). However, the factor time is not an experimental factor with levels randomized to subplot units. Instead, the levels must occur in sequence. When time is treated as a factor that can interact with other factors, the F test performed in the univariate analysis might not be valid (Littell, 1989). For the test to be valid, all the measurements must have the same variance and all pairs of measurements must have the same correlation (Snedecor and Cochran, 1980). When performing univariate analysis of variance, the above assumption is considered to hold true. This may not be true in the case where one of the factors in the analysis and its interaction with the other factors is time. Objectives of the studies reported here were to analyze the root growth response over time of lettuce seedlings to different environmental factors to detect a trend over time of root growth morphology and development as affected by the treatments. The effect of time on plant growth was not of major interest, for it is well known that plants grow over time. The effect of the environment under which the plant was grown was the focus of the research. Each plant was measured daily to plot a growth trend which would detect whether different root growth responses were due to the treatments. In this chapter, methods of the statistical analyses conducted throughout the research are considered.

Growing Material

Lettuce seedlings were grown as previously discussed under Photosynthetic Whole Plant Culture, PWPC (Chapter 4), or under Photo-Mixotrophic Whole Plant Culture, PMWPC (see Chapter 3) system. Daily measurements were made on each plant, and data were submitted to the analysis of variance of repeated measurements to investigate the plant growth response over time (Littell, 1989). Data were analyzed using the REPEATED statement with the GLM procedure in the SAS system (SAS Institute Inc., 1989).

Discussion

Correlation Between the Repeated Measures Within Plants

One requirement to perform the univariate analysis of variance (F test) is that all pairs of measurements on the same plants must have the same correlation (Snedecor and Cochran, 1980). These correlations are called partial correlations (Littell, 1989).

Correlation values between lateral root numbers were high in a study comparing seedlings grown in medium with or without sucrose with the PMWPC system, when time between measures was short (Appendix A8-1). For instance, partial correlation between lateral root numbers at day 4 and at day 5 was 0.78, between lateral root numbers at day 5 and at day 6 was 0.70. As the length of time between measures increased, partial correlation between measures decreased. For instance, partial correlation between lateral root numbers at day 4 and at day 6 was 0.45, between lateral root numbers at day 5 and at day 7 was 0.33. Also, partial correlation between day 4 and day 8 was 0.15, between day 4 and day 9 was 0.02.

Partial correlation coefficients between basal root numbers in the same study indicated a different trend (Appendix A8-2). Numbers of roots of day 4 were hardly correlated with numbers of any other day, regardless of the length of time between measurements. The partial correlation coefficients were never greater than 0.32. Greater correlation was found between basal root numbers at 5 and 6 DAS (0.64), between 6 and 7 DAS (0.71), between 6 and 8 DAS (0.89), and between 7 and 9 DAS (0.82). This can be due to the very small number of basal roots produced in seedlings at day 4 and 5, close to 0 and smaller than one. When basal root number increased, so did the partial correlations.

Partial correlations of total root numbers (Appendix A8-3) had similar trends as those of lateral root numbers. Taproot length presented high partial correlation coefficients throughout the time (Appendix A8-4). Correlation was 0.93 between day 4 and 5, 0.83 between day 4 and 6, 0.72 between day 4 and 7, 0.67 between day 4 and 8, 0.64 between day 4 and 9. Not only were partial correlations high between the taproot length in the early days and subsequent days, but also during the growing period. For instance, between day 6 and 7 the correlation was 0.95.

In a study described in Chapter 6, seedlings were grown until 13 DAS. Also in this study trends of partial correlations in lateral, basal and total root number decreased when the length of time between measures increased (Appendix A8-5,6,7). However, partial correlation coefficients were very small between measures taken during the first 2 days. After 6 days, correlations increased between measures taken one day or two days apart. When the length of time between measures increased (e.g., day 6 vs. day 10) partial correlations decreased again. As already mentioned, during the very early days of lettuce seedling growth, roots had few primary branches. Consequently, presence of zeros and ones affected the partial correlation coefficients.

In another study, seedlings were grown for 18 days after seeding (Chapter 6). After 5 days of seedling growth, partial correlation coefficients of lateral root numbers increased in measures taken 1 or 2 days apart, beginning 5 DAS. For example, number of lateral roots between 5 and 6 DAS had a partial correlation coefficient of 0.81, between 5 and 7 DAS the coefficient was 0.56. Correlations were very low between 4 DAS and any other day (0.20 between 4 and 5 DAS, 0.08 between 4 and 7 DAS). Only few seedlings differentiated lateral roots 4 DAS, whereas most of them began 5 DAS. Conversely, basal roots began to emerge 4 DAS in most of the seedlings (Chapter 6). This could explain why partial correlations between basal root counts were high since day 4 (e.g., 0.77 between 4 and 5 DAS, 0.71 between 4 and 6 DAS) (Appendix A8-9). Trends of correlations decreased when length of time between measures was more than 2 days.

The previously discussed data indicated that there was correlation between measures and that there was a decreasing trend in correlations as the length of time between measures increased. This peculiar behavior of the variables is typical of repeated measures and raises doubts about the satisfaction of the assumption made to analyze the data with the univariate analysis. If a univariate analysis of variance F tests is performed, the result is an inflated α value (an increase in the chance of Type I error).

Tests for Validity of Univariate Analysis of Variance

The condition that must be satisfied to analyze the data with univariate analysis, called the Huynh-Feldt (H-F) condition (Huynh and Feldt, 1970), can be tested with an approximate χ^2 test (Littell, 1989) and is available in SAS (Mauchly's criterion for orthogonal contrasts). Whenever this χ^2 approximation has a significant probability, it implies that a split-plot in time analysis cannot be performed. Consequently another type of analysis should be pursued, which does not require the Huynh-Feldt assumption.

Greenhouse-Geisser (G-G) epsilon and Huynh-Feldt (H-F) epsilon are indexes that also measure the validity of univariate analysis of variance: the smaller these indexes are, the greater is the adjustment to be made to the P value of the univariate analysis of variance (Littell, personal communication). The more that these indexes approach the value of one, the more valid is the univariate analysis of variance. Likewise, the more these indexes tend to be towards zero, the less valid is the univariate analysis of variance. Analysis of the approximate χ^2 tests (Mauchly's criterion for orthogonal contrasts) and G-G and H-F epsilons were performed for the data collected throughout the research (Chapters 3, 6 and 7). In any experiments presented the probabilities of the χ^2 approximation were significant, consequently failing the test to meet the H-F assumption. In addition, the G-G and H-F epsilons were always much lower than the value of one.

The Greenhouse-Geisser Test

Littell (1989) listed different solutions for analyzing data when the H-F condition does not hold, among which the use of multivariate analysis or the adjustment of the degrees of freedom of univariate F-test types of analysis. Littell suggested a compromise among the tests and the use of the Greenhouse-Geisser (G-G) (Greenhouse and Geisser, 1959) adjustment to the univariate P values. This test eventually gives a P value that according to Littell (1989) is probably more valid than the univariate or multivariate tests.

Validity of the F test for main plot treatment effects and their interaction with other main plot treatment effects does not require the Huynh-Feldt (1970) condition of repeated measurements (Littell, 1989), consequently the univariate F test can be considered correct. However, test statistics for factors interacting with time require the H-F condition to be met. Univariate F statistics with Greenhouse-Geisser adjustments for the factors interacting with time were used in the present research (Appendix A8-20 through 8-23). Some experiments had a split-plot experimental design for the investigated factors; some others were arranged in a randomized complete block design. In the ANOVA presented, the G-G-corrected P values were equal or greater than the univariate F test, since they were adjusted according to Greenhouse and Geisser (1959).

The Trend Analysis

The present studies reported in this dissertation considered time as an important variable in analyzing the response of lettuce seedling root growth to different environmental factors. Time, of course, was not a factor, therefore trends over time were thought to be an appropriate analysis, by assessing linear, quadratic and cubic components for interaction between time and the experimental factors. In a study comparing ten genotypes of lettuce grown with or without sucrose in the medium, polynomial trends were computed (Appendix A8-24). Genotype interacted with time and the time trend presented significant linear and quadratic components. Sucrose interacted with time, and the time trend presented significant linear, quadratic and cubic components. Genotype x Sucrose interacted with time, and the time trend presented significant linear, quadratic and cubic components.

One of the difficulties that arose from these analyses was to relate the significant results of the trends to a meaningful interpretation of the time trend. Another approach to a better interpretation of the time trend could be achieved by plotting the data over time and analyze whether differences existed among treatments in different phases of plant growth (Chapter 3, 6 and 7).

In a study (Chapter 3) comparing 10 genotypes of lettuce grown in test tubes with or without sucrose, collected data (which consisted of lateral, basal or total root number and taproot length of individual plants) were plotted over time (Figures 3-1 through 3-8). Data revealed agrowth pattern that could be distinguished in three phases. An initial phase (Phase 1, between 4 and 5 days after seeding, DAS) was characterized by a steady growth of the plant. A second phase (Phase 2, between 5 and 6 DAS) was characterized by an accelerated root growth. A third phase (Phase 3, between 6 and 9 DAS) was characterized by a steady continuation of root growth. Both the difference in growth between day 6 and 9 and the slope of the linear fitting curve were considered in the analysis.

The univariate F tests of the 3 phases were considered (Appendix A8-25). From these analyses, considerations on importance of different phases of root growth due to the treatments could be identified. For instance, genotype affected the initial (Phase 1) lateral and total root number, but not the basal root number nor taproot length. However, the presence of sucrose in the medium enhanced basal root formation in Phase 1. During Phase 2, genotype continued to determine lateral root formation, but to a lesser extent. Total root number in Phase 2 was no longer affected by genotype. From the analyses it could be noticed that exogenous sucrose application did not affect lateral and total root numbers in Phase 1, whereas it affected basal root number. Exogenous sucrose affected root architecture during Phase 2 and 3. These phase analyses permitted distinction between different patterns of lettuce root growth depending on the treatments, helping to understand which factors were more influential on the early root growth compared to the following growth period.

Further analysis can be accomplished by analyzing each significant phase with a mean separation method, such as the LSD, or Standard Errors can be computed. For instance, in the case of lateral root number, in each phase the interaction between the 2 factors under investigation, cultivar and sucrose, was not significant. Therefore, analysis on the main effects could be performed. Least Squares Means and their Standard Errors of the ten different genotypes were listed (Appendix A8-26) and variations among genotype identified. When an interaction between genotype and sucrose was significant, as in the case of basal root number in Phase 3, Least Squares Means and their Standard Errors were computed.

Types of fitting curves to analyze the trends might be computed, however, the nonlinear regression method would be involved (Littell, 1989). The methods above described could be used to interpret the data collected in the present research and to identify phases of root growth more affected by environmental conditions than other.

Summary

The split-plot in time design, with time as a subplot factor, has generally been used to analyze the response over time of treatments. However, the factor time is not an experimental factor with levels randomized to subplot units. When time is treated as a factor that can interact with other treatments, the F test performed in the univariate analysis might not be valid, if all the measurements do not have the same variance and all pairs of measurements do not have the same correlation. The above assumption (Huynh-Feldt, H-F assumption) may not be true in the case where one of the factors in the analysis and its interaction with the other factors is time. Data collected daily on the same sampling unit, a lettuce seedling, indicated that there was a correlation between measurements taken and that there was a decreasing trend in correlations as the length of time between measurements increased. This peculiar behavior is typical of repeated measures and raises doubts about the satisfaction of the assumption made to analyze the data with the univariate analysis. In any experiment, tests for the validity of the H-F assumption were highly significant, indicating that this assumption was not met, therefore another type of analysis was pursued. Data were plotted over time to detect whether different root growth responses were due to the treatments interacting with time. Adjustments of the degrees of freedom of univariate F-test types of analysis were made in the analysis over time, using the Greenhouse-Geisser (G-G) adjustment of the univariate P values. An approach to interpret the time interacting with the environmental conditions affecting seedling growth could be achieved by analyzing whether differences existed among treatments in different phases of plant growth. These phase analyses permitted distinctions of different patterns of lettuce root growth depending on the treatments, helping to understand which factors were more influencing the early root growth compared to the following growth period. Even though some methods required numerous computations, they were obtainable through SAS computer system.

CHAPTER 9
SUMMARY

Introduction

Early seedling root growth and development determine the optimum root system throughout the entire life of a plant, consequently affecting growth during this period, and potentially leading to optimization of yields (Leskovar and Stoffella, 1995; Lynch, 1995). The spatial distribution in the soil of the root system can determine the potential of a plant to exploit the soil's resources, which are unevenly distributed on the earth's surface, or subjected to localized depletion by the roots (Lynch, 1995). The production of a primary root system, i.e. the primary branching from the radicle, is an important phenomenon in growth and survival of a plant (MacIsaac et al., 1989). A primary root system increases the surface area available for the uptake of water and mineral elements. In addition, with its architecture, a primary root system provides physical support to the developing shoot. To understand the implications of the root system on lettuce seedling growth and subsequent plant development, studies relating the effects of the environment immediately after radicle protrusion were conducted.

Sucrose Effect on Primary Root Branch Formation of Different Lettuce Genotypes

Seedling root morphology has been described as differing between monocotyledonous and dicotyledonous species (Fahn, 1982; Leskovar and Stoffella, 1995; Sutton and Tinus, 1983; Zobel, 1975, 1986). Dicotyledonous species generally present four types of roots: radicle, adventitious, lateral and basal roots (Chapter 5). Differences between the types of root can be taken into account when investigating root function, growth, or development (Zobel, 1986). However, limited research on the variability of root systems among genotypes has been done with crop species (Seiler, 1994).

Wild species of lettuce may become adapted to stressful environments by producing more vigorous root systems. Current lettuce varieties were usually selected under more favorable growing conditions wherein root structure may have become less important to the plant as well the breeder. The wild progenitors of lettuce are potential sources for enhancing the root system of the species. A primary objective of the present research was to characterize the root architecture and development of young lettuce seedlings and its variability among genotypes. However, field methods to study root growth enabling repeated direct observations of the roots without their disruption have not been reported (Mackie-Dawson and Atkinson, 1991). Partial observation methods have been established with the use of viewing surface inserted into the soil, but extrapolation of the root measurements to the entire root system may be not representative.

A whole-plant observation method was used to study the early root growth and development of lettuce seedlings (Chapter 3). The experimental approach was based on the use of micropropagation. Phytagel, a transparent gelled medium supplied of mineral nutrients, was used to grow lettuce seedlings in glass vessels. Tubes were covered with loose caps but not sealed. Because growth of seedlings can be restricted in relatively airtight vessels where photosynthesis would presumably be limiting, exogenous addition of sucrose to the medium may be required (Kozai, 1991a). Sucrose provides carbon skeletons for biosynthetic and respiratory processes during plant growth (Farrar and Williams, 1991) and is readily used as a carbon skeleton by plants after enzymatic hydrolysis (Avigah, 1982; Giaquinta, 1983; Smith, 1993). Consequently, sucrose was supplied to the medium creating a Photo-Mixotrophic Whole Plant Culture (PMWPC) system.

Root architecture of young lettuce seedlings grown for 9 days after seeding (DAS) was evaluated in ten genotypes [8 selected cultivars and 2 wild plant introduction (PI) lines] of lettuce, either grown in medium with or without exogenous sucrose (Chapter 3). Sucrose feeding and genotypes affected lateral, basal and total number of roots, and taproot elongation. Variability among genotype was more pronounced during the early phases of seedling growth (4 to 6 DAS), whereas the effect of exogenous sucrose feeding began to play a major role in root growth and development later, 5 to 9 DAS. The PI lines produced more lateral roots than the selected cultivars when exogenous sucrose was not applied. Exogenous sucrose enhanced lateral root formation in all genotypes, but more in two PI lines, 'Valmaine' and 'Tall Guzmaine'. Sucrose increased the numbers of basal roots in all genotypes except 'Dark Green Boston'. 'Valmaine' produced more basal roots than any other genotype when sucrose was added. 'Dark Green Boston', which differentiated few primary branches, produced the longest taproot, 4 to 9 DAS. At harvest, sucrose increased shoot and root dry mass. All genotypes had increased shoot dry mass when sucrose was added in the medium, while 'Valmaine', 'PI 251245' and 'PI

68288' had the greatest shoot dry mass. Root to shoot dry mass ratio increased in lettuce seedlings when sucrose was added to the medium. The greatest root to shoot ratio was achieved by 'PI 251245'.

The study reported in Chapter 3 indicated that exogenously supplied sucrose increased plant growth of lettuce seedlings, affecting the partitioning of synthates between root and shoot, and differentially increased the formation of the primary root system between lateral and basal roots and among genotypes. Lettuce seedling growth in terms of root formation and biomass was restricted when sucrose was absent in the medium. Genetic variability for rooting characteristics was found in lettuce, both cultivated and wild genotypes, indicating that incorporating specific rooting patterns into new cultivars would be an effective mean of genetically selecting new lettuce biotypes.

Photosynthetic Whole Plant Culture (PWPC) System

A PMWPC system lacks sufficient airflow into the vessels, consequently requiring exogenous carbon supply to guaranty plant growth. Sucrose is usually the basic carbon supply for plant growth in heterotrophic tissue culture, however, seedlings can grow autotrophically if sufficient

CO_2 and light are provided (Kozai, 1989; Kozai et al., 1987, 1988a, 1988b, 1988c, 1991a). Consequently, the objective of a study reported in Chapter 4 was to design, build and test a Photosynthetic Whole Plant Culture (PWPC) system to study root seedling growth, which would allow non-destructive root measurements such as root counts and at the same time allow the direct observation and description of the root architecture of autotrophic plant culture lettuce seedlings.

Glass one-liter bottles were used to accommodate a large quantity of transparent nutrient medium, thus permitting the growth of seedlings for up to 18 DAS without supplying sucrose as a carbon source. Filtered and calibrated air was allowed to flow in and out the bottles, thus permitting the seedlings to use their photosynthetic capacity. Environmental conditions of the growth room such as temperature, relative humidity and light were preconditioned and controlled throughout the duration of the experiments.

The workability of the system was assessed by a series of tests aimed to establish which gelling agent resulted more translucent upon solidification of the autoclaved medium, and which prior-to-autoclaving medium pH was giving the desired after-autoclaving medium pH of 6±1. Phytagel gelling agent used at a concentration of 2.5 g l^{-1} and prior-to-autoclaving pH of 6.5 was determined to be an optimal growth support medium. Changes of medium pH over time as affected by seedling presence in the bottle and environmental conditions to be applied during the seedling growth studies (Chapter 6 and 7) were evaluated. The medium pH was only affected by the presence of the seedlings and restricted to the rhizosphere.

The growing system developed was easily constructed, calibrated and monitored during lettuce seedling root growth studies, and enabled direct non-destructive plant observations and measurements, under controlled environmental conditions. During the reported studies (Chapters 6 and 7) pregerminated seeds were transferred one into each bottle and grown until plant harvest. Number of lateral and basal roots, number of secondary root branches and of leaves could be daily counted, by visual observation through the bottle. The root growth observation method developed could represent a valuable tool to investigate root morphology and development in different species, to improve selection and breed new varieties based on their root architecture potential.

Root Morphology and Architecture in Young Lettuce Seedlings

Differences in root branch formation exist in many crops. Lettuce is a horticultural crop which has not been studied for its root architecture. Understanding whether differentiation between lateral and basal root formation occurs in lettuce may be valuable to improve growth rates of lettuce seedlings. Detailed information of the sequence of events occurring in root branch formation of seedlings grown in a controlled environment could be valuable to further determine the root development of field-grown plants. A root architectural analysis with a scanning electron microscope and a scanning image system was conducted on 'Valmaine' lettuce seedlings grown for 18 DAS (Chapter 5) enabling the determination of root differentiation and of the series of events involved in root development. Results indicated that 'Valmaine' lettuce seedlings produce two types of primary root branches, basal and lateral roots, as reported in other dicotyledonous species (Leskovar and Stoffella, 1995; Stoffella *et al.*, 1979; Stoffella et al., 1988; Zobel, 1975). Differentiation in primary branch formation between lateral and basal roots could be valuable for increased survival adaptation of lettuce seedlings to different soil conditions which can affect root resource acquisition potentiality. Seedlings did not produce adventitious roots, as was reported for bell pepper by Stoffella et al. (1988).

Basal and lateral roots in lettuce seedlings originated from two distinct regions of the taproot and developed differently. Basal roots originated at two opposite sides of a short upper portion of the taproot and located close to the hypocotyl, and did not produce secondary branches during the first 18 DAS. Lateral roots originated at about 120° apart along a longer portion of the taproot and produced secondary branches. This finding is an indication that lateral roots can explore more soil volume for resources than basal roots, thus lateral roots may be able to reach and exploit localized patches of nutrients in the soil (Lynch, 1995). Conversely, basal root bi-directional and superficial formation gives the root system an horizontal extension in the soil surface, assuring the basal roots the capacity to exploit the most fertile portions of agricultural soils (Bole, 1977; Eshel and Waisel, 1996).

Basal roots emerged prior to or at the same day as lateral roots and after full cotyledon expansion. Same results were reported by Stoffella et al. (1988) in pepper (*Capsicum annuum* L.) seedlings. However, lateral roots emerged prior to basal roots in tomato (*Lycopersicon esculentum* Mill.) seedlings (Aung, 1982).

The series of events in lettuce root development could be characterized as: a) early radicle elongation from radicle protrusion without any branching until cotyledons were unfolded; b) emergence of basal roots 4 DAS; c) emergence of lateral roots at the same day or later than basal roots, 4 or 5 DAS; d) increased acropetal emergence of basal roots and basipetal emergence of lateral roots in the following days, from 5 to 18 DAS; e) emergence of secondary branches 9 or 10 DAS and arising basipetally from the older lateral roots; f) emergence of basal and lateral roots between older primary roots, contemporary to emergence of basal and lateral roots that followed the original acropetal and basipetal patterns; g) formation of two- to threefold number of lateral roots compared to basal roots.

The study described in Chapter 5 indicated that lettuce seedlings form an elaborated root branch system. This has a dual influence on plant growth, both to increase the root surface area available for water and mineral uptake and to provide support to the developing shoot. This information could be useful when trying to genetically select lettuce cultivars for superior rooting patterns. The demonstration that lateral and basal roots are formed in lettuce might be valuable to determine the rooting patterns of greenhouse-

and field-grown plants and, thus, relate these to improved head quality and yield.

Influence of Carbon-source on Root Architecture and Development in Lettuce Seedlings

Exogenous application of sucrose in PMWPC systems is known to enhance plant growth. With the increasing finding on the role of sucrose in morphogenic signals (Aloni, 1987; Bernier, 1988; Bernier, 1996; Bernier *et al.*, 1993; Corbesier et al., 1996; Farrar and Williams, 1991; Jan and Sheen, 1996; Koch, 1996; Koch *et al.*, 1996; Kovtun and Daie, 1995), interpretation of results from experiments using sucrose in the medium must be looked very carefully. Kovtun and Daie (1995) reported that exogenous sucrose application led to altered sink-to-source status of leaves, because seedlings could use carbon supply through photosynthetic activity. Several lines of evidence support the hypothesis that the path of C entry into a plant system or organ can alter its effect at both the substrate and signaling levels. These alterations may be related to altered effects on growth and morphology.

In the studies reported in Chapter 6, PWPC has been used to address the hypotheses that a lettuce root system can differentially respond to sucrose feeding and to free air flow, and further, that plant response to sucrose feeding may vary compared to C provided by CO_2 in the atmosphere. Results of this work had dual significance, both to applied systems for production and screening of seedlings with extensive root systems, and to fundamental studies of sugar effects on plant growth as influenced by endogenous versus exogenous paths of sucrose delivery.

'Valmaine' lettuce seedlings were grown for 13 DAS, either with or without sucrose. Analysis of root growth and development revealed that sucrose-fed seedlings produced more lateral, basal and total roots than nonsucrose-fed seedlings during the early days of plant growth (4 to 7 DAS). After that, exogenous sucrose did not affect root branch formation. At 13 DAS, root dry mass of lettuce seedlings was similar between treatments. Sucrose-fed seedlings had greater shoot dry mass than nonsucrose-fed seedlings.

In a second study, 'Valmaine' lettuce seedlings were grown for 18 days. Plants were either supplied with or without sucrose and with 350 $\mu mol\ mol^{-1}$ CO_2 (standard air) or 2000 $\mu mol\ mol^{-1}$ CO_2 (CO_2-enriched air). Analysis of root growth and development revealed that CO_2 enrichment enhanced lateral root formation from 4 to 7 DAS, basal root formation from 4 to 18 DAS and secondary branch formation in nonsucrose-fed seedlings. Exogenous application of sucrose enhanced root branch formation during the early days of seedlings growth, but after that exogenous sucrose suppressed lateral primary and secondary root formation. At 18 DAS, seedling dry biomass did not differ among the treatments. Thus, exogenous sucrose application to the seedlings and CO_2 enrichment in the air altered root branch formation, without affecting plant biomass.

Collectively, the two studies reported in Chapter 6 indicated that a differential morphological response due to the path of C entry into a plant occurred in lettuce seedling root growth, and did not alter plant biomass, besides a greater shoot dry mass occurred 13 DAS in the first study. Increased CO_2 concentration in the atmosphere enhanced lettuce root formation. Using CO_2 enrichment in controlled environment would be a valuable approach to increase early root branching in lettuce seedlings during greenhouse transplant production. The present research added another evidence in supporting the hypothesis that the path of C entry into a plant system can alter the plant growth itself differently.

Carbon Dioxide Enrichment and Nitrogen Effects on Lettuce Root Growth and Development

Increased concentration of CO_2 in the atmosphere has received increased interest in recent years (Eshel and

Waisel, 1996). Plant adaptation to rising CO_2 concentrations in the atmosphere is a paramount priority these days, because it is estimated that CO_2 in the atmosphere will double from the 1978 average concentration of 338 $\mu mol\ mol^{-1}$ by the middle or later part of the 21^{st} century (Murray, 1995). Enrichment of atmospheric CO_2 has been reported to increase growth rate and yield of a wild variety of plants, and it is widely used in greenhouse crop production. However, the effect of elevated CO_2 in the atmosphere has often been studied by scientists as related to shoot growth.

In a previous study, CO_2 enrichment was reported to increase root branch formation in lettuce seedlings (Chapter 6). The ecological and physiological implications of such altered root morphology and architecture are related to water and nutrient acquisition capacity from soil of the root system (Fitter, 1985; Fitter and Stickland, 1991; Lynch and van Beem, 1993). Long et al. (1996) reported that acclimatory loss of Rubisco activity and protein due to CO_2 enrichment occurred in seedlings of *Lolium perenne* grown at two N levels, considered limiting and saturating. However, the acclimation was more evident when N was limiting. Johnson et al. (1996) reported that plant growth was precluded by N deficiency in *Pinus* seedlings grown under elevated CO_2. Net photosynthesis has been reported to increase linearly with increasing plant concentrations of organic N under saturating light conditions (Mooney and Winner, 1991). Increasing N supply, however, could change root morphology and development to allow the roots to adapt to the changed conditions. This hypothesis is based on the fact that in the short-term, plants tend to increase their NO_3 uptake when NO_3 supply is increased, thus affecting root growth (Burns, 1991; Drew and Saker, 1975; Edwards and Barber, 1976; Robinson and Rorison, 1983).

The effects on root morphology and development of the association between the activities of C and N assimilatory processes were evaluated in a study described in Chapter 7. A PWPC system was used to grow 'Valmaine' lettuce seedlings for 18 DAS and to determine root architectural changes as affected by CO_2 enrichment and N supply. Plants were supplied with either 60 mM or 120 mM of N as NH_4NO_3 and KNO_3 in the medium. Two concentrations of CO_2 were applied, 350 $\mu mol\ mol^{-1}$ CO_2 (standard atmospheric air), or 2000 $\mu mol\ mol^{-1}$ CO_2 (CO_2-enriched air). Results indicated that CO_2 enrichment affected lettuce seedling root architecture, as it did in another study (Chapter 6), by enhancing root branch formation. This was more evident when 60 mM N was supplied. When 120 mM N was added to the medium, root branch formation was reduced in seedlings grown with normal air. Root morphological changes due to N supply did not alter plant biomass at harvest. An increased biomass partitioning towards the roots was detected in seedlings grown with CO_2-enriched air. The study indicated that increased CO_2 availability, although did not lead to an increased plant growth in terms of biomass, probably because an acclimation effect occurred in the photosynthetic rate (Evans, 1983, 1987; Geiger and Servaites, 1991; Kramer, 1981; Sage et al., 1989; Sonnewald et al., 1996; Torisky and Servaites, 1984), altered root architecture of lettuce seedlings. However, increased CO_2 availability did not lead to an increased N plant demand. On the contrary, increased levels of N supply reduced root branch formation.

Collectively, results from the study indicated that CO_2 and N treatments may not alter plant biomass of lettuce seedlings, but root morphological changes in the root system occur, which can alter its architecture for the entire plant life. An elaborated branch formation obtained with elevated CO_2 concentration and reduced N supply can increase the soil volume occupied by the roots, enhancing their soil exploration capacity and their support of the shoot to the soil, thus improving seedling stability and establishment in the field. Enhancing lettuce root growth is one of the goals for researchers and growers during greenhouse transplant production, to increase field stand establishment of seedlings. If information from the present research would be confirmed in greenhouse-grown seedlings, improved root branch systems of lettuce transplants might be achieved by reducing N applications and increasing CO_2 concentration in greenhouse-controlled atmosphere.

Statistical Analysis of Repeated Measurements to Investigate Root Growth Development

One of the objectives of studies reported in the present dissertation was to analyze the plant root growth response over time of lettuce seedlings to different environmental factors in order to detect a trend over time of root growth morphology and development as affected by treatment. The effect of time on plant growth was not of major interest, for it is well known that plants grow over time. It was the effect of the environment under which the plant was grown which was the focus of the research. Each plant was measured daily in order to plot a growth trend which would detect whether different root growth responses were due to treatment. The common approach to the analysis of response over time of treatment has usually been the 'split-plot in time design' (Gomez and Gomez, 1984). However, the factor time is not an experimental factor with levels randomized to subplot units. Instead, the levels must occur in sequence. When time is treated as a factor that can interact with other treatments, the F test performed in the univariate analysis might not be valid, if all the measurements do not have the same variance and all pairs of measurements do not have the same correlation (Littell, 1989; Snedecor and Cochran, 1980). In performing an univariate analysis of variance the above assumption (Huynh-Feldt, H-F assumption; Huynh and Feldt, 1970) may not be true in the case where one of the factors in the analysis and its interaction with the other factors is time. Consequently, data of repeated measures from the present dissertation were tested for the validity of the univariate analysis of variance and alternative statistic methods to interpret the results were implemented.

Data collected daily on the same sampling unit, a lettuce seedling, indicated that there was a correlation between taken measures and that there was a decreasing trend in correlations as the length of time between measures increased. This peculiar behavior is typical of repeated measures and arises doubts about the satisfaction of the assumption made to analyze the data with the univariate analysis. In every experiments, tests for the validity of the H-F assumption were highly significant, indicating that this assumption was not met. Consequently another type of analysis was pursued. Data were plotted over time to detect whether different root growth responses were due to the treatments interacting with time. Adjustments of the degrees of freedom of univariate F-test types of analysis were made in the analysis over time, using the Greenhouse-Geisser (G-G) adjustment of the univariate P values (Greenhouse and Geisser, 1959). An approach to interpret the time interacting with the environmental conditions affecting seedling growth could be achieved by analyzing whether differences existed among treatments in different phases of plant growth. These phase analyses permitted distinctions of different patterns of lettuce root growth depending on the treatment, helping to understand which factors were more influencing the early root growth compared to the following growth period.

The statistical approach used to analyze data in interaction with time was considered appropriate since mathematical conditions required for validity of split-plot in time design were not met. The usually overlooked assumptions to be met when performing univariate analysis of variance become particularly important in the case of split-plot in time designs. The present study demonstrated that analyses of plant growth over time should not be performed with the F tests, otherwise, validity of the consequent interpretations of the results could be dubious. Even though some methods required numerous computations, they were obtained thank to modern computer programs.

Future Research

Findings from the present research certainly stimulate ideas for further research on root systems. Differential rooting patterns found in lettuce genotypes could be investigated at the gene expression level, seeking to identify the gene(s) involved in basal and lateral root initiation. The enhancement in root branch formation obtained when CO_2 was enriched could be further investigated to clarify whether sucrose translocation to the root system occurred, and if so, how it induced morphogenic changes in the roots without affecting biomass. Possible hormone involvement, such as auxin and cytokinin, could be further studied to understand the signal transduction pathway of sugars. Nitrogen metabolism is well understood in plants. However, further studies could be conducted to explain the inhibiting role of N on root formation. Different concentrations of N in the seedling growth medium could be tested to appraise the extent that reduced N application would enhance root branch formation without limiting shoot growth. Other environmental factors could be studied. For instance, studies on the effects on root development of increased light intensity when CO_2 is enriched may be valuable. Seedlings could be grown under different photoperiods to maximize root development. Mineral nutrients, such as K, P and Ca, play an important role in plant growth, and their effects could be evaluated on root morphogenesis using the PWPC system. Gradient supply of nutrients in the rhizosphere would allow the researcher to better understand the role played by basal and lateral roots on nutrient acquisition by the plant.

APPENDIX A
STATISTICAL TABLES

Appendix A8-1. Partial correlations between lateral root numbers (Lat4-Lat9) at day 4 through 9 of experiment in test tubes (Chapter 3).

	Lat4	Lat5	Lat6	Lat7	Lat8	Lat9
Lat4	---	0.78	0.45	0.25	0.15	0.02
Lat5	0.78	---	0.70	0.33	0.14	-0.05
Lat6	0.45	0.70	---	0.64	0.37	0.10
Lat7	0.25	0.33	0.64	---	0.80	0.55
Lat8	0.15	0.14	0.37	0.80	---	0.90
Lat9	0.02	-0.05	0.10	0.55	0.90	---

Appendix A8-2. Partial correlations between basal root numbers (Bas4-Bas9) at day 4 through 9 of experiment in test tubes (Chapter 3).

	Bas4	Bas5	Bas6	Bas7	Bas8	Bas9
Bas4	---	0.32	0.32	0.23	0.23	0.16
Bas5	0.32	---	0.64	0.46	0.45	0.35
Bas6	0.32	0.64	---	0.71	0.72	0.56
Bas7	0.23	0.46	0.71	---	0.89	0.82
Bas8	0.23	0.45	0.72	0.89	---	0.91
Bas9	0.16	0.35	0.56	0.82	0.91	---

Appendix A8-3. Partial correlations between total root numbers (Tot4-Tot9) at day 4 through 9 of experiment in test tubes (Chapter 3).

	Tot4	Tot5	Tot6	Tot7	Tot8	Tot9
Tot4	---	0.72	0.48	0.27	0.21	0.05
Tot5	0.72	---	0.71	0.35	0.20	0.00
Tot6	0.48	0.71	---	0.66	0.44	0.17
Tot7	0.27	0.35	0.66	---	0.82	0.60
Tot8	0.21	0.20	0.44	0.82	---	0.90
Tot9	0.05	0.00	0.17	0.60	0.90	---

Appendix A8-4. Partial correlations between taproot length (Taplth4-Taplth9) at day 4 through 9 of experiment in test tubes (Chapter 3).

	Taplth4	Taplth5	Taplth6	Taplth7	Taplth8	Taplth9
Taplth4	---	0.93	0.83	0.72	0.67	0.64
Taplth5	0.93	---	0.94	0.86	0.80	0.76
Taplth6	0.83	0.94	---	0.95	0.89	0.83
Taplth7	0.72	0.86	0.95	---	0.97	0.91
Taplth8	0.67	0.80	0.89	0.97	---	0.97
Taplth9	0.64	0.76	0.83	0.91	0.97	---

Appendix A8-5. Partial correlations between lateral root numbers (Lat4-Lat13) at day 4 through 13 of the first experiment in bottles (Chapter 6).

	Lat4	Lat5	Lat6	Lat7	Lat8	Lat9	Lat10	Lat11	Lat12	Lat13
Lat4	---	0.41	0.33	0.15	0.01	-0.16	-0.03	-0.13	-0.15	-0.13
Lat5	0.41	---	0.67	0.51	0.38	0.38	0.27	0.24	0.15	0.11
Lat6	0.33	0.67	---	0.77	0.67	0.42	0.39	0.36	0.26	0.24
Lat7	0.15	0.51	0.77	---	0.83	0.58	0.59	0.59	0.52	0.51
Lat8	0.01	0.38	0.67	0.83	---	0.71	0.71	0.72	0.61	0.54
Lat9	-0.16	0.38	0.42	0.58	0.71	---	0.88	0.88	0.84	0.77
Lat10	-0.03	0.27	0.39	0.59	0.71	0.88	---	0.96	0.93	0.88
Lat11	-0.13	0.24	0.36	0.59	0.72	0.88	0.96	---	0.97	0.92
Lat12	-0.15	0.15	0.26	0.52	0.61	0.84	0.93	0.97	---	0.97
Lat13	-0.13	0.11	0.24	0.51	0.54	0.77	0.88	0.92	0.97	---

Appendix A8-6. Partial correlations between basal root numbers (Bas4-Bas13) at day 4 through 13 of the first experiment in bottles (Chapter 6).

	Bas4	Bas5	Bas6	Bas7	Bas8	Bas9	Bas10	Bas11	Bas12	Bas13
Bas4	---	0.52	0.37	0.34	0.13	0.00	0.12	0.09	0.04	-0.11
Bas5	0.52	---	0.88	0.80	0.58	0.57	0.62	0.61	0.58	0.47
Bas6	0.37	0.88	---	0.78	0.52	0.48	0.53	0.56	0.55	0.44
Bas7	0.34	0.80	0.78	---	0.68	0.62	0.64	0.64	0.62	0.54
Bas8	0.13	0.58	0.52	0.68	---	0.89	0.79	0.80	0.81	0.70
Bas9	0.00	0.57	0.48	0.62	0.89	---	0.89	0.89	0.91	0.83
Bas10	0.12	0.62	0.53	0.64	0.79	0.89	---	0.93	0.90	0.81
Bas11	0.09	0.61	0.56	0.64	0.80	0.89	0.93	---	0.95	0.92
Bas12	0.04	0.58	0.55	0.62	0.81	0.91	0.90	0.95	---	0.94
Bas13	-0.11	0.47	0.44	0.54	0.70	0.83	0.81	0.92	0.94	---

Appendix A8-7. Partial correlations between total root numbers (Tot4-Tot13) at day 4 through 13 of the first experiment in bottles described in Chapter 6.

	Tot4	Tot5	Tot6	Tot7	Tot8	Tot9	Tot10	Tot11	Tot12	Tot13
Tot4	---	0.51	0.39	0.30	0.15	0.05	0.21	0.14	0.08	0.02
Tot5	0.51	---	0.66	0.59	0.56	0.58	0.47	0.41	0.33	0.24
Tot6	0.39	0.66	---	0.78	0.70	0.46	0.40	0.36	0.30	0.24
Tot7	0.30	0.59	0.78	---	0.83	0.61	0.61	0.59	0.54	0.47
Tot8	0.15	0.56	0.70	0.83	---	0.78	0.66	0.67	0.61	0.51
Tot9	0.05	0.58	0.46	0.61	0.78	---	0.88	0.86	0.83	0.74
Tot10	0.21	0.47	0.40	0.61	0.66	0.88	---	0.95	0.91	0.85
Tot11	0.14	0.41	0.36	0.59	0.67	0.86	0.95	---	0.97	0.92
Tot12	0.08	0.33	0.30	0.54	0.61	0.83	0.91	0.97	---	0.97
Tot13	0.02	0.24	0.24	0.47	0.51	0.74	0.85	0.92	0.97	---

Appendix A8-8. Partial correlations between lateral root numbers (Lat4-Lat18) at day 4 through 18 of the second experiment in bottles (Chapter 6).

	Lat4	Lat5	Lat6	Lat7	Lat8	Lat9	Lat10	Lat11	Lat12	Lat13	Lat14	Lat15	Lat16	Lat17	Lat18
Lat4	---	0.20	0.08	-0.06	0.00	0.10	0.08	-0.03	-0.06	-0.09	-0.08	-0.11	-0.11	-0.05	-0.07
Lat5	0.20	---	0.81	0.56	0.47	0.32	0.29	0.17	0.20	0.18	0.27	0.30	0.22	0.20	0.16
Lat6	0.08	0.81	---	0.74	0.62	0.46	0.46	0.39	0.35	0.30	0.32	0.34	0.28	0.26	0.23
Lat7	-0.06	0.56	0.74	---	0.85	0.61	0.57	0.54	0.48	0.42	0.46	0.45	0.48	0.46	0.45
Lat8	0.00	0.47	0.62	0.85	---	0.81	0.74	0.70	0.69	0.61	0.65	0.58	0.60	0.58	0.57
Lat9	0.10	0.32	0.46	0.61	0.81	---	0.89	0.81	0.81	0.71	0.72	0.62	0.67	0.66	0.67
Lat10	0.08	0.29	0.46	0.57	0.74	0.89	---	0.96	0.92	0.81	0.76	0.63	0.65	0.66	0.67
Lat11	-0.03	0.17	0.39	0.54	0.70	0.81	0.96	---	0.94	0.85	0.78	0.66	0.68	0.69	0.70
Lat12	-0.06	0.20	0.35	0.48	0.69	0.81	0.92	0.94	---	0.95	0.91	0.81	0.79	0.80	0.81
Lat13	-0.09	0.18	0.30	0.42	0.61	0.71	0.81	0.85	0.95	---	0.96	0.85	0.79	0.78	0.79
Lat14	-0.08	0.27	0.32	0.46	0.65	0.72	0.76	0.78	0.91	0.96	---	0.94	0.89	0.88	0.87
Lat15	-0.11	0.30	0.34	0.45	0.58	0.62	0.63	0.66	0.81	0.85	0.94	---	0.96	0.95	0.93
Lat16	-0.11	0.22	0.28	0.48	0.60	0.67	0.65	0.68	0.79	0.79	0.89	0.96	---	0.99	0.96
Lat17	-0.05	0.20	0.26	0.46	0.58	0.66	0.66	0.69	0.80	0.78	0.88	0.95	0.99	---	0.98
Lat18	-0.07	0.16	0.23	0.45	0.57	0.67	0.67	0.70	0.81	0.79	0.87	0.93	0.96	0.98	---

Appendix A8-9. Partial correlations between basal root numbers (Bas4-Bas18) at day 4 through 18 of the second experiment in bottles (Chapter 6).

	Bas4	Bas5	Bas6	Bas7	Bas8	Bas9	Bas10	Bas11	Bas12	Bas13	Bas14	Bas15	Bas16	Bas17	Bas18
Bas4	---	0.77	0.71	0.51	0.35	0.42	0.47	0.32	0.34	0.32	0.29	0.29	0.32	0.32	0.27
Bas5	0.77	---	0.84	0.53	0.33	0.40	0.46	0.35	0.38	0.40	0.38	0.39	0.43	0.43	0.42
Bas6	0.71	0.84	---	0.61	0.39	0.46	0.51	0.41	0.42	0.45	0.42	0.45	0.46	0.47	0.46
Bas7	0.51	0.53	0.61	---	0.82	0.75	0.72	0.68	0.71	0.70	0.65	0.62	0.63	0.63	0.60
Bas8	0.35	0.33	0.39	0.82	---	0.83	0.76	0.77	0.82	0.80	0.77	0.71	0.70	0.69	0.65
Bas9	0.42	0.40	0.46	0.75	0.83	---	0.88	0.86	0.87	0.82	0.78	0.71	0.72	0.73	0.72
Bas10	0.47	0.46	0.51	0.72	0.76	0.88	---	0.95	0.89	0.84	0.80	0.73	0.75	0.77	0.76
Bas11	0.32	0.35	0.41	0.68	0.77	0.86	0.95	---	0.89	0.84	0.82	0.75	0.78	0.79	0.79
Bas12	0.34	0.38	0.42	0.71	0.82	0.87	0.89	0.89	---	0.95	0.92	0.85	0.88	0.88	0.88
Bas13	0.32	0.40	0.45	0.70	0.80	0.82	0.84	0.84	0.95	---	0.96	0.91	0.91	0.90	0.89
Bas14	0.29	0.38	0.42	0.65	0.77	0.78	0.80	0.82	0.92	0.96	---	0.95	0.95	0.95	0.93
Bas15	0.29	0.39	0.45	0.62	0.71	0.71	0.73	0.75	0.85	0.91	0.95	---	0.96	0.95	0.92
Bas16	0.32	0.43	0.46	0.63	0.70	0.72	0.75	0.78	0.88	0.91	0.95	0.96	---	0.99	0.97
Bas17	0.32	0.43	0.47	0.63	0.69	0.73	0.77	0.79	0.88	0.90	0.95	0.95	0.99	---	0.99
Bas18	0.27	0.42	0.46	0.60	0.65	0.72	0.76	0.79	0.88	0.89	0.93	0.92	0.97	0.99	---

Appendix A8-10. Partial correlations between total root numbers (Tot4-Tot18) at day 4 through 18 of the second experiment in bottles (Chapter 6).

	Tot4	Tot5	Tot6	Tot7	Tot8	Tot9	Tot10	Tot11	Tot12	Tot13	Tot14	Tot15	Tot16	Tot17	Tot18
Tot4	---	0.75	0.67	0.42	0.34	0.37	0.39	0.26	0.30	0.25	0.27	0.34	0.36	0.37	0.31
Tot5	0.75	---	0.85	0.66	0.58	0.53	0.51	0.40	0.46	0.45	0.47	0.50	0.46	0.46	0.43
Tot6	0.67	0.85	---	0.74	0.63	0.59	0.57	0.48	0.48	0.45	0.45	0.45	0.44	0.44	0.43
Tot7	0.42	0.66	0.74	---	0.87	0.78	0.72	0.68	0.68	0.65	0.69	0.64	0.64	0.64	0.62
Tot8	0.34	0.58	0.63	0.87	---	0.86	0.83	0.80	0.83	0.80	0.84	0.77	0.72	0.73	0.71
Tot9	0.37	0.53	0.59	0.78	0.86	---	0.92	0.86	0.87	0.83	0.85	0.81	0.78	0.79	0.77
Tot10	0.39	0.51	0.57	0.72	0.83	0.92	---	0.97	0.91	0.85	0.85	0.79	0.76	0.78	0.77
Tot11	0.26	0.40	0.48	0.68	0.80	0.86	0.97	---	0.93	0.86	0.84	0.77	0.74	0.76	0.76
Tot12	0.30	0.46	0.48	0.68	0.83	0.87	0.91	0.93	---	0.97	0.95	0.91	0.87	0.87	0.87
Tot13	0.25	0.45	0.45	0.65	0.80	0.83	0.85	0.86	0.97	---	0.97	0.90	0.85	0.84	0.84
Tot14	0.27	0.47	0.45	0.69	0.84	0.85	0.85	0.84	0.95	0.97	---	0.95	0.92	0.91	0.91
Tot15	0.34	0.50	0.45	0.64	0.77	0.81	0.79	0.77	0.91	0.90	0.95	---	0.97	0.96	0.95
Tot16	0.36	0.46	0.44	0.64	0.72	0.78	0.76	0.74	0.87	0.85	0.92	0.97	---	0.99	0.98
Tot17	0.37	0.46	0.44	0.64	0.73	0.79	0.78	0.76	0.87	0.84	0.91	0.96	0.99	---	0.99
Tot18	0.31	0.43	0.43	0.62	0.71	0.77	0.77	0.76	0.87	0.84	0.91	0.95	0.98	0.99	---

Appendix A8-11. Partial correlations between lateral branches (Br10-Br18) at day 4 through 18 of the second experiment in bottles (Chapter 6).

	Br10	Br11	Br12	Br13	Br14	Br15	Br16	Br17	Br18
Br10	---	0.82	0.65	0.60	0.52	0.42	0.14	0.12	0.09
Br11	0.82	---	0.76	0.69	0.59	0.49	0.27	0.23	0.19
Br12	0.65	0.76	---	0.96	0.89	0.76	0.61	0.59	0.56
Br13	0.60	0.69	0.96	---	0.95	0.85	0.71	0.67	0.59
Br14	0.52	0.59	0.89	0.95	---	0.95	0.78	0.73	0.63
Br15	0.42	0.49	0.76	0.85	0.95	---	0.84	0.80	0.69
Br16	0.14	0.27	0.61	0.71	0.78	0.84	---	0.98	0.89
Br17	0.12	0.23	0.59	0.67	0.73	0.80	0.98	---	0.96
Br18	0.09	0.19	0.56	0.59	0.63	0.69	0.89	0.96	---

Appendix A8-12. Partial correlations between lateral root numbers (Lat4-Lat18) at day 4 through 18 of experiment in bottles (Chapter 7).

	Lat4	Lat5	Lat6	Lat7	Lat8	Lat9	Lat10	Lat11	Lat12	Lat13	Lat14	Lat15	Lat16	Lat17	Lat18
Lat4	---	0.10	-0.04	0.00	0.28	0.26	0.40	0.20	-0.01	-0.11	-0.20	0.01	0.05	0.13	0.04
Lat5	0.10	---	0.63	0.34	0.63	0.29	0.23	0.31	0.29	0.17	0.06	0.15	0.20	0.09	0.13
Lat6	-0.04	0.63	---	0.74	0.74	0.58	0.24	0.29	0.39	0.32	0.19	0.39	0.39	0.37	0.39
Lat7	0.00	0.34	0.74	---	0.71	0.59	0.02	0.00	0.15	0.08	0.28	0.29	0.28	0.40	0.49
Lat8	0.28	0.63	0.74	0.71	---	0.67	0.36	0.39	0.43	0.34	0.32	0.34	0.33	0.24	0.29
Lat9	0.26	0.29	0.58	0.59	0.67	---	0.61	0.47	0.56	0.40	0.51	0.56	0.36	0.28	0.27
Lat10	0.40	0.23	0.24	0.02	0.36	0.61	---	0.89	0.68	0.56	0.47	0.64	0.37	0.24	0.17
Lat11	0.20	0.31	0.29	0.00	0.39	0.47	0.89	---	0.83	0.75	0.57	0.77	0.54	0.33	0.30
Lat12	-0.01	0.29	0.39	0.15	0.43	0.56	0.68	0.83	---	0.93	0.74	0.84	0.56	0.35	0.28
Lat13	-0.11	0.17	0.32	0.08	0.34	0.40	0.56	0.75	0.93	---	0.70	0.80	0.44	0.25	0.18
Lat14	-0.20	0.06	0.19	0.28	0.32	0.51	0.47	0.57	0.74	0.70	---	0.82	0.61	0.48	0.40
Lat15	0.01	0.15	0.39	0.29	0.34	0.56	0.64	0.77	0.84	0.80	0.82	---	0.78	0.68	0.61
Lat16	0.05	0.20	0.39	0.28	0.33	0.36	0.37	0.54	0.56	0.44	0.61	0.78	---	0.90	0.79
Lat17	0.13	0.09	0.37	0.40	0.24	0.28	0.24	0.33	0.35	0.25	0.48	0.68	0.90	---	0.92
Lat18	0.04	0.13	0.39	0.49	0.29	0.27	0.17	0.30	0.28	0.18	0.40	0.61	0.79	0.92	---

Appendix A8-13. Partial correlations between basal root numbers (Bas4-Bas18) at day 4 through 18 of experiment in bottles (Chapter 7).

	Bas4	Bas5	Bas6	Bas7	Bas8	Bas9	Bas10	Bas11	Bas12	Bas13	Bas14	Bas15	Bas16	Bas17	Bas18
Bas4	---	0.05	0.25	0.51	0.33	0.57	0.68	0.61	0.54	0.50	0.43	0.24	0.20	0.19	0.17
Bas5	0.05	---	0.21	0.11	0.02	-0.12	0.06	0.09	0.14	0.01	0.06	0.10	0.29	0.38	0.35
Bas6	0.25	0.21	---	0.78	0.77	0.69	0.58	0.50	0.47	0.47	0.53	0.46	0.52	0.49	0.42
Bas7	0.51	0.11	0.78	---	0.85	0.80	0.71	0.58	0.47	0.46	0.50	0.36	0.48	0.39	0.45
Bas8	0.33	0.02	0.77	0.85	---	0.84	0.63	0.52	0.42	0.42	0.42	0.38	0.51	0.40	0.43
Bas9	0.57	-0.12	0.68	0.80	0.84	---	0.81	0.72	0.56	0.58	0.59	0.48	0.50	0.47	0.52
Bas10	0.68	0.06	0.58	0.71	0.63	0.81	---	0.88	0.76	0.73	0.72	0.52	0.52	0.38	0.40
Bas11	0.61	0.09	0.50	0.58	0.52	0.72	0.88	---	0.92	0.88	0.92	0.74	0.72	0.63	0.60
Bas12	0.54	0.14	0.47	0.47	0.42	0.56	0.76	0.92	---	0.98	0.93	0.81	0.78	0.68	0.56
Bas13	0.50	0.01	0.47	0.46	0.42	0.58	0.73	0.88	0.98	---	0.93	0.84	0.77	0.66	0.56
Bas14	0.43	0.06	0.53	0.50	0.42	0.59	0.72	0.92	0.93	0.93	---	0.90	0.84	0.77	0.68
Bas15	0.24	0.10	0.46	0.36	0.38	0.48	0.52	0.74	0.81	0.84	0.90	---	0.94	0.87	0.79
Bas16	0.20	0.29	0.52	0.48	0.51	0.50	0.52	0.72	0.78	0.77	0.84	0.94	---	0.92	0.84
Bas17	0.20	0.38	0.49	0.39	0.40	0.47	0.38	0.63	0.68	0.66	0.77	0.87	0.92	---	0.91
Bas18	0.17	0.35	0.42	0.45	0.43	0.52	0.40	0.60	0.56	0.56	0.68	0.79	0.84	0.90	---

Appendix A8-14. Partial correlations between total root numbers (Tot4-Tot18) at day 4 through 18 of experiment in bottles (Chapter 7).

	Tot4	Tot5	Tot6	Tot7	Tot8	Tot9	Tot10	Tot11	Tot12	Tot13	Tot14	Tot15	Tot16	Tot17	Tot18
Tot4	---	0.36	0.07	0.20	0.37	0.46	0.54	0.42	0.38	0.35	0.21	0.44	0.47	0.52	0.44
Tot5	0.36	---	0.68	0.50	0.62	0.44	0.47	0.49	0.54	0.47	0.33	0.33	0.19	0.19	0.16
Tot6	0.07	0.68	---	0.67	0.69	0.53	0.33	0.31	0.38	0.33	0.14	0.27	0.30	0.30	0.18
Tot7	0.20	0.50	0.67	---	0.73	0.55	0.27	0.17	0.23	0.17	0.25	0.12	0.26	0.31	0.47
Tot8	0.37	0.62	0.69	0.73	---	0.73	0.57	0.53	0.49	0.45	0.42	0.40	0.51	0.38	0.46
Tot9	0.46	0.44	0.53	0.55	0.73	---	0.79	0.66	0.62	0.55	0.64	0.56	0.54	0.49	0.46
Tot10	0.54	0.47	0.33	0.27	0.57	0.79	---	0.89	0.79	0.73	0.74	0.77	0.62	0.51	0.48
Tot11	0.42	0.49	0.31	0.17	0.53	0.66	0.89	---	0.87	0.81	0.81	0.88	0.66	0.49	0.44
Tot12	0.38	0.54	0.38	0.23	0.49	0.62	0.79	0.87	---	0.96	0.85	0.85	0.59	0.40	0.22
Tot13	0.35	0.47	0.33	0.17	0.45	0.55	0.73	0.81	0.96	---	0.83	0.82	0.52	0.30	0.11
Tot14	0.21	0.33	0.14	0.25	0.42	0.64	0.74	0.81	0.85	0.83	---	0.78	0.52	0.30	0.25
Tot15	0.44	0.33	0.27	0.12	0.40	0.56	0.77	0.88	0.85	0.82	0.78	---	0.76	0.63	0.43
Tot16	0.47	0.19	0.30	0.26	0.51	0.54	0.62	0.66	0.59	0.52	0.52	0.76	---	0.84	0.62
Tot17	0.52	0.19	0.30	0.31	0.38	0.49	0.51	0.49	0.40	0.30	0.30	0.63	0.84	---	0.81
Tot18	0.44	0.16	0.18	0.47	0.46	0.46	0.48	0.44	0.22	0.11	0.25	0.43	0.62	0.81	---

Appendix A8-15. Partial correlations between lateral branches (Br9-Br14) at day 9 through 14 of experiment in bottles (Chapter 7).

	Br9	Br10	Br11	Br12	Br13	Br14
Br9	---	0.89	0.83	0.61	0.20	0.27
Br10	0.89	---	0.92	0.72	0.46	0.44
Br11	0.83	0.92	---	0.83	0.66	0.64
Br12	0.61	0.72	0.83	---	0.80	0.69
Br13	0.20	0.46	0.66	0.80	---	0.84
Br14	0.27	0.44	0.64	0.69	0.84	---

Appendix A8-16. Results of the approximate χ^2 test performed on the data collected in the study in test tubes (Chapter 3).

	Root number			Length
	Lateral	Basal	Total	Taproot
Test for Sphericity (Mauchly's Criterion)	5.90E-03	8.16E-02	1.16E-02	2.03E-02
χ^2 approximation	1006.41	491.30	873.47	764.62
P of χ^2 approximation	0.0000	0.0000	0.0000	0.0000
Greenhouse-Geisser Epsilon	0.3408	0.4510	0.3589	0.4409
Huynh-Feldt Epsilon	0.3937	0.5233	0.4151	0.5113

Appendix A8-17. Results of the approximate χ^2 test performed on the data collected in the first study in bottles (Chapter 6).

	Root number			Number of	
	Lateral	Basal	Total	Leaves	Branches
Test for Sphericity					
(Mauchly's Criterion)	2.25E-05	3.29E-04	2.86E-05	1.74E-02	1.01E-01
χ^2 approximation	243.92	182.84	238.46	92.39	52.78
P of χ^2 approximation	0.0000	0.0000	0.0000	0.0000	0.0000
Greenhouse-Geisser Epsilon	0.2319	0.2631	0.2440	0.5487	0.5260
Huynh-Feldt Epsilon	0.3205	0.3691	0.3392	0.8752	0.7049

Appendix A8-18. Results of the approximate χ^2 test performed on the data collected in the second study in bottles (Chapter 6).

	Root number			Number of
	Lateral	Basal	Total	Branches
Test for Sphericity (Mauchly's Criterion)	1.94E-10	5.35E-11	9.62E-11	3.27E-07
χ^2 approximation	674.02	712.90	695.23	479.711
P of χ^2 approximation	0.0000	0.0000	0.0000	0.0000
Greenhouse-Geisser Epsilon	0.1865	0.1233	0.1487	0.2264
Huynh-Feldt Epsilon	0.2259	0.1439	0.1764	0.2654

Appendix A8-19. Results of the approximate χ^2 test performed on the data collected in the study in bottles (Chapter 7).

	Root number			Number of
	Lateral	Basal	Total	Branches
Test for Sphericity				
(Mauchly's Criterion)	1.60E-07	8.92E-11	9.54E-07	2.60E-03
χ^2 approximation	205.66	304.13	182.19	95.83
P of χ^2 approximation	0.0000	0.0000	0.0000	0.0000
Greenhouse-Geisser Epsilon	0.3272	0.2255	0.3040	0.3129
Huynh-Feldt Epsilon	0.5745	0.3550	0.5204	0.4326

Appendix A8-20. Univariate analysis of variance of repeated measures data over time of experiment in test tubes (Chapter 3).

		Lateral Roots		Basal Roots		Total Roots		Taproot Length	
		Univariate	G - G	Univariate	G - G	Univariate	G - G	Univariate	G - G
Source of variation	DF	F Test	Adjustment	F Test	Adjustment	F Test	Adjustment	F Test	Adjustment
Block	1								
Genotype	9	0.0001		0.0135		0.0004		0.0143	
Genotype * Block (error a)	9								
Sucrose	1	0.0001		0.0002		0.0001		0.0008	
Sucrose * Genotype	9	0.0062		0.2640		0.0140		0.4953	
Sucrose * Block (Genotype) (error b)	9								
Sampling error	189								
Analysis over Time									
Time	5	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	<0.0001	0.0001
Time * Block	5	0.0001	0.0008	0.0079	0.0306	0.0001	0.0019	0.0005	0.0096
Time * Genotype	45	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Time * Genotype * Block	45	0.0277	0.1172	0.0446	0.1018	0.0021	0.0378	0.0001	0.0020
Time * Sucrose	5	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Time * Genotype * Sucrose	45	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0040
Error	990								

Appendix A8-21. Univariate analysis of variance of repeated measures data over time of the first study in bottles (Chapter 6).

		Lateral Roots		Basal Roots		Total Roots			Lateral Branches	
Source of variation	DF	Univariate F Test	G - G Adjust.	Univariate F Test	G - G Adjust.	Univariate F Test	G - G Adjust.		Univariate F Test	G - G Adjust.
Block	3									
Sucrose	1	0.2418		0.0718		0.1585			0.2188	
Error	3									
Sampling error	26									
Analysis over time								DF		
Time	9	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	3	0.0001	0.0001
Time * Block	27	0.0460	0.1774	0.0209	0.1260	0.0129	0.1137	9	0.0001	0.0035
Time * Sucrose	9	0.0001	0.0015	0.0148	0.0948	0.0001	0.0005	3	0.0014	0.0105
Error	234							78		

Appendix A8-22. Univariate analysis of variance of repeated measures data over time of the second study in bottles (Chapter 6).

Source of variation	DF	Lateral Roots		Basal Roots		Total Roots			Lateral branches	
		Univariate F Test	G - G Adjustment	Univariate F Test	G - G Adjustment	Univariate F Test	G - G Adjustment		Univariate F Test	G - G Adjustment
Block	1									
CO_2	1	0.0084		0.2163		0.0873			0.0043	
Sucrose	1	0.0074		0.9350		0.3222			0.0226	
Sucrose * CO_2	1	0.1652		0.8123		0.8998			0.0261	
Error	3									
Sampling error	32									
Analysis over Time								DF		
Time	14	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	8	0.0001	0.0001
Time * Block	14	0.0001	0.0153	0.0001	0.0272	0.0001	0.0057	8	0.7483	0.5183
Time * CO_2	14	0.0044	0.0897	0.0001	0.0173	0.0001	0.0067	8	0.0001	0.0228
Time * Sucrose	14	0.0001	0.0001	0.1980	0.2753	0.0001	0.0001	8	0.0457	0.1469
Time * CO_2 * Sucrose	14	0.5976	0.4496	0.9989	0.7718	0.9465	0.6319	8	0.1630	0.2352
Error	490							280		

Appendix A8-23. Univariate analysis of variance of repeated measures data over time of study in bottles (Chapter 7).

		Lateral Roots		Basal Roots		Total Roots		Lateral branches	
Source of variation	DF	Univariate F Test	G - G Adjustment	Univariate F Test	G - G Adjustment	Univariate F Test	G - G Adjustment	Univariate F Test	G - G Adjustment
Block	1								
CO_2	1	0.1421		0.1644		0.1495		0.1403	
CO_2 * Block (error a)	1								
Nitrogen	1	0.0087		0.0757		0.0208		0.2027	
CO_2 * Nitrogen	1	0.0742		0.2145		0.1099		0.7110	
Nitrogen * Block (CO_2) (error b)	2								
Sampling error	16								
Analysis over Time									
Time	14	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Time * Block	14	0.2576	0.3060	0.0002	0.0303	0.0109	0.0809	0.9147	0.6932
Time * CO_2	14	0.0043	0.0519	0.0001	0.0119	0.0001	0.0001	0.0027	0.0395
Time * CO_2 *Block	14	0.0458	0.1371	0.8882	0.6466	0.0019	0.0420	0.7964	0.5822
Time * Nitrogen	14	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0040	0.0456
Time * CO_2 * Nitrogen	14	0.1574	0.2396	0.0015	0.0572	0.0002	0.0182	0.5818	0.4473
Error	252							df=90	

Appendix A8-24. Analyses of variance for polynomial time trends performed on the data collected in the study in test tubes (Chapter 3).

Variable: Lateral Roots

Source of variation	DF	MS	F	P value
Time (Linear)	1	1625.51	243.37	0.0001
Time (Quad)	1	5.62	2.48	0.1168
Time (Cubic)	1	13.11	22.36	0.0001
Time (Linear) x Genotype	9	45.74	6.85	0.0001
Time (Quad) x Genotype	9	5.08	2.24	0.0209
Time (Cubic) x Genotype	9	0.47	0.80	0.6198
Time (Linear) x Sucrose	1	1223.06	183.12	0.0001
Time (Quad) x Sucrose	1	37.04	16.35	0.0001
Time (Cubic) x Sucrose	1	20.28	34.59	0.0001
Time (Linear) x Genotype * Sucrose	9	31.55	4.72	0.0001
Time (Quad) x Genotype * Sucrose	9	4.40	1.94	0.0484
Time (Cubic) x Genotype * Sucrose	9	1.17	1.99	0.0422
Error	198			

Variable: Basal Roots

Source of variation	DF	MS	F	P value
Time (Linear)	1	141.59	137.49	0.0001
Time (Quad)	1	0.33	1.23	0.2683
Time (Cubic)	1	0.22	1.63	0.2038
Time (Linear) x Genotype	9	5.12	4.97	0.0001
Time (Quad) x Genotype	9	0.41	1.53	0.1405
Time (Cubic) x Genotype	9	0.12	0.89	0.5310
Time (Linear) x Sucrose	1	106.64	103.56	0.0001
Time (Quad) x Sucrose	1	2.78	10.42	0.0015
Time (Cubic) x Sucrose	1	0.01	0.04	0.8483
Time (Linear) x Genotype * Sucrose	9	5.24	5.09	0.0001
Time (Quad) x Genotype * Sucrose	9	0.73	2.75	0.0048
Time (Cubic) x Genotype * Sucrose	9	0.06	0.42	0.9259
Error	198			

Appendix A8-24--continued

Variable: Total Roots				
Source of variation	DF	MS	F	P value
Time (Linear)	1	2726.59	329.52	0.0001
Time (Quad)	1	3.23	1.18	0.2788
Time (Cubic)	1	16.75	20.35	0.0001
Time (Linear) x Genotype	9	67.26	8.13	0.0001
Time (Quad) x Genotype	9	5.83	2.13	0.0290
Time (Cubic) x Genotype	9	0.60	0.73	0.6778
Time (Linear) x Sucrose	1	2052.01	248.00	0.0001
Time (Quad) x Sucrose	1	60.09	21.92	0.0001
Time (Cubic) x Sucrose	1	20.92	25.41	0.0001
Time (Linear) x Genotype * Sucrose	9	48.00	5.80	0.0001
Time (Quad) x Genotype * Sucrose	9	7.25	2.65	0.0065
Time (Cubic) x Genotype * Sucrose	9	1.24	1.51	0.1479
Error	198			

Variable: Lateral Branches				
Source of variation	DF	MS	F	P value
Time (Linear)	1	148113.93	2371.77	0.0001
Time (Quad)	1	3640.74	138.46	0.0001
Time (Cubic)	1	109.16	11.01	0.0011
Time (Linear) x Genotype	9	544.13	8.71	0.0001
Time (Quad) x Genotype	9	159.17	6.05	0.0001
Time (Cubic) x Genotype	9	9.55	0.96	0.4715
Time (Linear) x Sucrose	1	12804.99	205.05	0.0001
Time (Quad) x Sucrose	1	4.33	0.16	0.6854
Time (Cubic) x Sucrose	1	254.88	25.71	0.0001
Time (Linear) x Genotype * Sucrose	9	139.04	2.23	0.0218
Time (Quad) x Genotype * Sucrose	9	75.35	2.87	0.0034
Time (Cubic) x Genotype * Sucrose	9	5.34	0.54	0.8449
Error	198			

Appendix A8-25. P values of the different segments of trend analysis of lateral, basal and total number of roots and taproot length of lettuce seedlings DAS 4 through 9 (Chapter 3).

	Lateral Roots				Basal Roots			
	Phase 1	Phase 2	Phase 3	Phase 3	Phase 1	Phase 2	Phase 3	Phase 3
Treatments			dif 9-6[x]	slopes			dif 9-6	slopes
Genotype	0.0041	0.0375	0.0278	0.0351	0.3476	0.1917	0.0629	0.0948
Sucrose	0.5946	0.0001	0.0001	0.0001	0.0170	0.0079	0.0001	0.0001
Sucrose * Genotype	0.9836	0.5909	0.0534	0.0656	0.3154	0.6427	0.0001	0.0001

	Total Roots				Taproot length			
	Phase 1	Phase 2	Phase 3	Phase 3	Phase 1	Phase 2	Phase 3	Phase 3
Treatments			dif 9-6[x]	slopes			dif 9-6	slopes
Genotype	0.0052	0.0961	0.0109	0.0132	0.3062	0.2988	0.1542	0.1741
Sucrose	0.1027	0.0001	0.0001	0.0001	0.2574	0.0008	0.0001	0.0001
Sucrose * Genotype	0.6565	0.6732	0.0242	0.0289	0.1558	0.9830	0.3510	0.3431

[x] Dif 9-6 indicates that the actual difference of the values between 6 and 9 DAS has been used for the analysis.
Slope indicates that the slope of the linear fitting curve has been used.

Appendix A8-26. Least Squares Means for the 10 genotypes of lettuce in lateral root number during the three phases of growth (Main effect significant at the ANOVA) (Chapter 3).

	Lateral Roots							
	Phase 1		Phase 2		Phase 3		Phase 3	
	(dif[x] 5-4)		(dif 6-5)		(dif 9-6)		(slope)	
Genotype	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Floribibb	0.59	0.18	0.74	0.26	1.94	0.96	0.31	0.17
Dark Green Boston	0.23	0.27	0.73	0.39	1.24	1.28	0.18	0.22
Parris Island Cos	0.07	0.25	0.57	0.37	2.69	1.21	0.44	0.21
Tall Guzmaine	0.05	0.19	0.33	0.27	3.16	0.99	0.52	0.17
Everglades	0.15	0.19	0.83	0.28	2.26	1.00	0.38	0.17
Floricos 83	0.44	0.16	0.66	0.23	0.93	0.88	0.15	0.15
Valmaine	0.41	0.17	1.13	0.24	4.18	0.91	0.68	0.16
Musette	0.07	0.17	0.31	0.24	1.76	0.91	0.30	0.16
PI 251245	0.39	0.19	1.04	0.27	4.73	0.97	0.78	0.17
PI 68288	1.15	0.16	1.57	0.22	3.04	0.87	0.50	0.15

[x] Dif indicates that the actual difference of the values betweeen the two points in time has been used for the analysis. Slope indicates that the slope of the linear fitting curve has been used.

Appendix A8-27. Least Squares Means of the slopes for the interaction of 10 genotypes of lettuce and sucrose in the medium effects in basal root number during Phase 3 of growth (Interaction significant at the ANOVA) (Chapter 3).

	Basal Roots		
	Phase 3 slopes		
Genotype	Sucrose ($g\ l^{-1}$)	LSM	SE
Floribibb	0	0.00	0.06
Floribibb	30	0.11	0.04
Dark Green Boston	0	0.00	0.06
Dark Green Boston	30	0.07	0.09
Parris Island Cos	0	0.01	0.06
Parris Island Cos	30	0.34	0.08
Tall Guzmaine	0	0.01	0.05
Tall Guzmaine	30	0.38	0.05
Everglades	0	0.00	0.06
Everglades	30	0.11	0.05
Floricos 83	0	0.00	0.04
Floricos 83	30	0.21	0.04
Valmaine	0	0.01	0.05
Valmaine	30	0.50	0.05
Musette	0	0.00	0.05
Musette	30	0.16	0.04
PI 251245	0	0.00	0.06
PI 251245	30	0.14	0.05
PI 68288	0	0.01	0.04
PI 68288	30	0.22	0.04

APPENDIX B
EXPERIMENTS ON SUCROSE AND CO_2: STUDY WITH SUCROSE IN THE MEDIUM AND CO_2-ENRICHED AIR

Table B-1. Overall means for significant effects at the Analysis of Variance.

		Number of			
		Lateral		Branches	
Treatments		P	Mean	P	Mean
CO_2 (µmol mol^{-1})	Sucrose (g l^{-1})				
CO_2		0.0084		0.0043	
350			10.02		3.19
2000			12.28		6.58
Sucrose		0.0074		0.0226	
	0		8.90		5.83
	30		14.98		3.95
CO_2 * Sucrose		0.1652		0.0261	
350	0				3.24
350	30				2.61
2000	0				7.34
2000	30				4.76

APPENDIX C
EXPERIMENT ON CO_2 AND NITROGEN

Table C-1. Overall means for N significant effect at the Analysis of Variance.

	Root Number			
	Lateral		Total	
Treatments	P	Mean	P	Mean
Nitrogen	0.0087		0.0208	
60 mM		18.23		26.44
120 mM		13.75		18.57

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BIOGRAPHICAL SKETCH

Silvana Nicola was born in Carignano, Torino, Italy, on November 5, 1961. In February 1988, she received her Laurea degree from the Universitá di Torino, Torino, Italy, and in November 1988, her Agronomist degree from the same university. In March 1988 she became a research assistant in the Dipartimento di Agronomia, Selvicoltura e Gestione del Territorio, Universitá di Torino. During the six years of her appointment at the Universitá di Torino, she conducted research related to seedling growth and development of vegetable species, and she published her results in national and international scientific journals. In November 1992, she was awarded a Fulbright Scholarship to spend six months in the United States of America to engage in research at the University of Florida in the Horticultural Sciences Department. While she was at the University of Florida, she was offered a research assistantship to pursue a doctoral program in the Horticultural Sciences Department. After a country-wide selection, in February 1996 Silvana has obtained a faculty position at the Universitá di Torino, Italy, and in September 1996 she accepted the position. Silvana is a member of the International Society for Horticultural Science, American Society for Horticultural Science, Societá Orticola Italiana, Hydroponic Society of America and Florida State Horticultural Society. She is author or coauthor to date of thirty scientific publications, including abstracts and papers.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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